




Sugar Pills? Investigating Humphreys' Homeopathic Specifics: Utilizing Liquid Chromatography-Tandem Mass Spectrometry for an In-Depth Look at Nineteenth-Century American Homeopathy

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Abstract

Frederick K. Humphreys (1816–1900), founder of Humphreys Medicine Company, appeared at the rise of both greater acceptance and popularity of the homeopathy in the United States, as opposed to more orthodox medicine. Although bolstered by the relatively high cost and low success rate of traditional medicines, homeopathic remedies soon became a source of controversy due to the largely unknown ingredient lists and contradictory claims of effectiveness. Tandem mass spectrometry analysis (MS/MS) was used to pinpoint the ingredients of four “specifics” in Humphreys' Homeopathic Remedies. Generated spectra showed similar base peak ions for each pill, leading to the hypothesis that each remedy contained the same basic ingredients. Further MS/MS analyses identified these base peak ions as adducts of sucrose (m/z 365, 381), its related ions (m/z 433, 203, 185), and apigenin (m/z 271). When placed into a historical context, the application of chemical analyses to study these early remedies can provide insight into the question of validity that evoked arguments during the late nineteenth century.

Key Words Homeopathy · Homoeopathic specifics · Frederick Humphreys · Mass spectrometry · Chemical analysis

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Introduction

Homeopathy entered the American medical landscape almost two centuries ago (Haller 2015:3-5). The principles of American homeopathy were based on the teachings of German physician, Samuel Hahnemann. More commonly referred to as the “philosophy of healing,” this alternative approach to medicine was fueled as an unconventional, yet more sensible recourse to often harmful medical techniques, including bloodletting and purgatives (Kuzniar 2017: 6, 15). The promotion of these more rigorous treatments, often prescribed in large doses, by traditional physicians promoted a sense of distrust and disbelief toward these practices, and when coupled with high costs, ultimately caused many individuals and families to turn to alternative treatment methods (Kuzniar 2017: 108). The origin of Hahnemann’s claims was based on the general absence of chemistry among practicing physicians. Hahnemann filled this void with a vigorous application of the science during trials for his own remedies (Gumpert 1945: 51-67). Homeopathic practices were further enhanced by Romanticism, which served as an ideal gateway to individualized care in treatment (Gumpert 1945: 18; Kuzniar 2017: 10). Initially, this philosophy was built around the use of a single medication to treat all symptoms afflicting a patient using only minimal doses, however, in practice, these principles continued to evolve (Haller 2015:9-37).

The initial concept of homeopathy grew in response to Hahnemann’s formal medical training in Vienna, where he became critical of certain practices he labeled as falsehoods, specifically questioning the side effects of a treatment in a healthy person (Haller 2015:10-11). As a response, Hahnemann began practicing a process called “proving,” which utilized treatments for an ailment that would evoke the same ailment, to a lesser degree, in a healthy person (Fishbein 1925: 33-36). This practice, frequently called the “law of similars,” was presented by Hahnemann (1796) in his “Essay on a New Principle for Ascertaining the Curative Power of Drugs” (1796), where he claimed that “every powerful medicinal substance produces in the human body a kind of peculiar disease; the more powerful the medicine, the more peculiar, marked and violent the disease.” Throughout his original learnings, Hahnemann looked toward nature to produce a similar “artificial disease”, in order to cure an initial chronic disease (Loudon 2006: 607-608). Hahnemann’s theory was further supported by his own independent trials prescribing medicines based the law of similars, as well as outside experiments, including Edward Jenner’s successful use of cowpox vaccination to prevent against the development of smallpox (Kuzniar 2017: 134).

Hahnemann heavily relied on nature throughout the development of homeopathy, drawing inspiration for both correct treatment path and as a source for his treatments. Hahnemann’s early medications were composed of vegetables mixed with alcohol, however overtime he identified sugar globules as the superior form of administration, believing that sugar aided the medicine in reaching its full potential (Loudon 2006: 607-608). Although in practice, homeopathy varied immensely in terms of patient and treatment approaches, the most controversial portion of Hahnemann’s teachings was centered in dosages. It was common

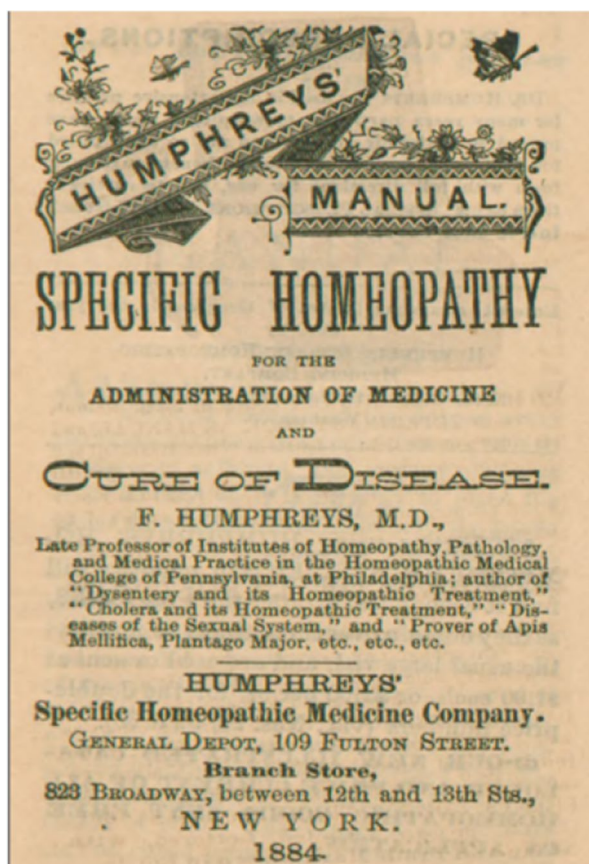
practice in prescribing homeopathic remedies that a patient was only to be given the smallest of amounts, in order to inflict only the slightest of side effects (Kuzniar 2017: 107). His recommended dilution ratio would be 1: 100000000, however Hahnemann claimed the remedies maintained effectiveness through a process of “potentization” that existed upon the basis of a “dematerialized spiritual force” if the remedy was shaken violently during preparation (Loudon 2006: 608). These theories drew directly upon what Hahnemann considered to be the purpose of a physician, “to restore health in a mild, prompt and durable manner” (Gumpert 1945: 137). Met with intense criticism, this marked the most controversial of Hahnemann’s claims, as well as the primary focal point of heightened scrutiny toward homeopathy, and Hahnemann as a physician, that continued to torture him until his death in 1843 (Gumpert 1945: 144-174). As a result, the basis of dilutions on a spiritual force caused a deep divide with scientific rigor causing many practicing homeopaths to adopt their own prescribing principles, often based on patient criteria and/or need (Fishbein 1925: 37).

Specifically, American homeopathy had been shaped by the same social, scientific, and philosophical forces as other schools of the healing art, leading to this alternative medicine to be regarded in a “friendlier” light by society (Fishbein 1925: 36-40). As a result, the popularity of homeopathy grew exponentially during the latter portion of the nineteenth century, especially in middle- to upper-class families who recognized the lack of evidence for orthodox medical treatments published in early medical and scientific texts (Haller 2015:5, 39-57).

Practitioners of homeopathy originally aimed to treat symptoms based on the totality of disease, choosing to focus on the underlying cause to determine an appropriate course of treatment. Addressing the root of the affliction was often easier when compared to conventional allopathy (Haller 2015:231-256). Through most of the nineteenth century, licensed physicians, lay practitioners, and self-care advocates practiced homeopathy. Contrary to the often dubbed “quacks,” homeopathic specialists came from diverse backgrounds, mirroring the standards of traditional medicine, as many homeopaths were licensed physicians or other certified medical professionals (Jarvis 2000). As this alternative treatment grew customary, commercial pamphlets and academic texts aided the increasing popularity. Notably, works such as *Family Homeopathy* (1864) and *The American Family Physician: Or, Domestic Guide to Health* (1864), as shown in Fig. 1, emerged to enlighten parents on how best to treat their children using homeopathic remedies, marketed as cures for both sudden onset and long-term diseases (Humphreys 1884). Coupled with the arrival of commercially available homeopathic solutions, including Humphreys’ Specifics, the principles behind this ideology began to evolve in order to accommodate more scientific dogma.

Frederick K. Humphreys (1816-1900), a homeopathic physician, quickly became a titan in the field as the demand for his products grew in the northeastern United States (Humphreys 1910). He founded Humphreys’ Homeopathic Medicine Co. in New York City in 1853, manufacturing and selling remedies for commercial use that were advertised to treat a multitude of ailments (Humphreys 1910). His specifics gained notoriety for being the first form of commercially available and effective homeopathic treatments, boasting an efficiency rate of five-sixths, and were featured

Fig. 1 Humphreys' homeopathic treatment guide marketed specifically to families in the mid-to-late nineteenth century, claiming upward of one hundred thousand customers, to use as an at-home alternative to traditional allopathic practices.



in popular newspapers and periodicals such as *The Philadelphia Inquirer* and *The Editor & Publisher and The Journalist* (The *Philadelphia Inquirer* 1875, 1915). An example of such advertising is shown in Fig. 2; an 1857 ad for Humphreys' Specifics in *The Philadelphia Inquirer* listing the remedies as "mild power cures" alongside the array of ailments they treated as they were primarily marketed for their simplicity, safety and convenience (*Philadelphia Inquirer* 1875). As an aside, these advertisements and ones similarly published during the origin of these remedies are congruent with the time period of the samples analyzed here.

Alongside practicing, Humphreys (1847) also authored doctrines of homeopathy including "Homeopathy vs. Allopathy" in *The American Journal of Homeopathy*. Published internationally, Humphreys' article detailed the precise preparatory methods of each remedy aiming to treat a specific disorder, ranging from kidney disease to diphtheria, through the combination of sugar for taste and vegetable medicines (Humphreys 1847:141). Additionally, Humphreys rallied against the lack of individualization in allopathic practices that homeopathy was able to provide for each patient and case (Humphreys 1884).

Fig. 2 Advertisement from *The Philadelphia Inquirer* for Humphreys' Homeopathic Specifics (1857).

MEDICAL.

THE MILD POWER CURES.—HUMPHREYS' HOMEOPATHIC SPECIFICS have proved, from the most ample experience, an entire success. Simple, prompt, efficient and reliable. They are the only medicines perfectly adapted to popular use.

Nos.	Cures.	Cents.
1	FEVERS, Congestion, Inflammations.....	25
2	WORMS, Worm Fever, Worm Colic.....	25
3	CRYING COLIC or Teething of Infants.....	25
4	DIARRHŒA of Children or Adults.....	25
5	DYSENTERY, Griping, Billious Colic.....	25
6	CHOLERA MORBUS, Vomiting.....	25
7	COUGHS, Colds, Bronchitis.....	25
8	NEURALGIA, Toothache, Faceache.....	25
9	HEADACHES, Sick Headache, Vertigo.....	25
10	DYSPEPSIA, Billious Stomach.....	25
11	SUPPRESSED or Painful Periods.....	25
12	WHITES, too Profuse Periods.....	25
13	CROUP, Cough, Difficult Breathing.....	25
14	SALT RHEUM, Erysipelas, Eruptions.....	25
15	RHEUMATISM, Rheumatic Pains.....	25
16	FEVER AND AGUE, Chills, Fever, Agues.....	50
17	PILES, Blind or Bleeding.....	50
18	OPHTHALMY, and Sore or Weak Eyes.....	50
19	CATARH, Acute or Chronic, Influenza.....	50
20	WHOOPIING COUGH, Violent Coughs.....	50
21	ASTHMA, Oppressed Breathing.....	50
22	EAR DISCHARGES, Impaired Hearing.....	50
23	SCROFULA, Enlarged Glands, Swellings.....	50
24	GENERAL DEBILITY, Physical Weakness.....	50
25	DROPSY and Scanty Secretions.....	50
26	SEA SICKNESS and sickness from riding.....	50
27	KIDNEY DISEASE, Gravel.....	60
28	NERVOUS DEBILITY, Vital Weakness.....	1'00
29	SORE MOUTH, Canker.....	50
30	URINARY WEAKNESS, Incontinence.....	50
31	PAINFUL PERIODS, with Spasms.....	50
32	SUFFERINGS at Change of Life.....	1'00
33	EPILEPSY, Spasms, St. Vitus Dance.....	1'00
34	DIPHThERIA, Ulcerated Sore Throat.....	50
35	CHRONIC CONGESTIONS and Eruptions.....	50
	Vials, 50 cents (except 28, 32, 33.).....	1'00

No. **FAMILY CASES.**

1	With 35 Large Three Drachm Vials, Rosewood Case, and Humphreys' Homeopathic Mentor (New Book).....	\$12'00
2	With 35 Large Three Drachm Vials, Morocco Case, and Specific Homeopathic Manual (small Book).....	10'00
3	With 29 Large Three Drachm Vials, Morocco Case, and Specific Homeopathic Manual.....	6'00

Sent by mail or express, free, on receipt of the price.

HUMPHREYS' WITCH HAZEL.

The indispensable Family Medicine for the cure of Piles, Burns or Scalds, Toothache, Neuralgia, Rheumatism, Bleedings, Ulcerations, Sores, Boils, Stings, Corps, Chilblains, etc. Price, 50c., \$1 and \$1'75 for different sizes. Witch Hazel Oil, the sure cure for Piles, &c. Price, 50c. Address

HUMPHREYS' HOMEOPATHIC MEDICINE CO.,
No. 562 BROADWAY, NEW YORK

The use of homeopathy was further supported by both patient testimonies and mortality rates of the time. Homeopathic Mutual Life Insurance Company (1868) offered discounted prices to patients of homeopathic medicine, resultant of the decreased mortality rates (Haller 2015: 98-115). A study by Dr. E. M. Kellogg for the aforementioned insurance company showed that, on average, for every ten homeopathy patients lost, 17 allopathy patients were lost to the same condition (Haller 2015:115). Overall, the average mortality rate for patients treated by orthodox medicine was 16.73% versus 9.74% for homeopathic treatments (Haller 2015:115). These promising statistical data were additionally backed by patient testimonies, often published in popular journals, including *The Republican Journal*, throughout the latter part of the nineteenth century and into the early twentieth century (*Republican Journal* 1922).

Despite the once great foothold homeopathy had in American households, the appeal of the medicine began to decline. Strong evidence for scientific theory arose in the early twentieth century, leading to attempts of homeopathic ideology reformation based on these developing principles. The reformation was futile, however, as the demand for homeopathy continued to diminish. Homeopathy itself was ridiculed in *The Medical Follies* (1925) not for its failure to heal, but for its faction-like principles and traditions that ultimately prevented the practice from being recognized as a legitimate medical specialty (Fishbein 1925). Fishbein (1925:41) asserted that “homeopathy died from within” after 1900 as graduates from schools of homeopathy began dwindling to just forty-nine in 1923. More so, a 1925 *New York Times Book Review* (1925) summarized these alternatives medicines as, “evolved by advocates of preposterous and fallacious theories, some of which were conceived for the purpose of deliberate fraud and others in the uncurbed delusions of fanatics.” These failures and resultant shift in public perspective resulted in fines and allegations of false advertisement in the 1920s, with Humphreys himself being criticized in 1918 and ultimately fined \$25 [Judgement No. 5635, New York] for false and fraudulent advertisement, as shown in Fig. 3 (Cramp 1921; *Journal of the American Medical Association* 1919:14-26).

Furthered by laws stemming from the creation of the Food and Drug Administration in 1906, companies were faced with this harsh scrutiny, primarily those based on homeopathic principles (Haller 2015:201). Allopathic physicians began an onslaught of slanderous attacks against the everchanging principles of homeopathy, as the original doctrines established by Hahnemann disappeared from the practices of more modern homeopaths (Haller 2015:201-230, 271). Utilizing this deviation to their advantage, the biomedical community refused to recognize homeopathy as a legitimate medical specialty solely based on sect, regardless of effectiveness to heal (Haller 2015:271-294).

Humphreys' Pile Ointment Witch Hazel Oil (Compound).—Shipped by Humphreys' Homeopathic Medicine Co., New York City. Analysis showed the preparation to be essentially a camphor ointment on a lard base. Falsely and fraudulently advertised. Fine, \$25.—[*Notice of Judgment No. 5635; issued April 29, 1918.*]

Fig. 3 Fine issued against Humphreys' Homeopathic Medicine Co. for false advertising associated with their Witch Hazel Oil in 1918.¹¹

Samuel H. Adams (1906:22-23) explained the growing instance of fake testimonials that often appeared as full-page advertisements in *The Great American Fraud: The Patent Medicine Evil*, “as for the men who permitted the use of their names for this purpose, several of them afterward admitted that they had never tasted the ‘Compound,’ but they were willing to sign the testimonials for the joy of appearing in print as “prominent citizens.” As a basis of commonality, only two Chicago-based newspapers were found to be free from this form of fake advertisements (Adams 1906: 23-24). The protective laws from the United States Food and Drug Administration (FDA) unintentionally aided this decline, as homeopathic companies faced accusations of false advertisement when forced to provide the public with a list of ingredients for each remedy (*Journal of the American Medical Association* 1919:14-26). Alternatively, the earlier homeopathic remedies (i.e., Humphreys’ Homeopathic specifics used in this study) are unhindered of this requirement, adding to the allure of investigating the original ingredients used at the height of American homeopathy.

Modern studies performed by Australia’s National Health and Medical Research Council (NHMRC) oversaw a systematic review of over 2,000 articles that evaluated the effects of homeopathic treatments on a variety of disorders and found that only 225 were conducted with enough viable scientific integrity to be properly appraised. Their conclusion was that no scientific correlation existed between homeopathic remedies and the effectiveness of treatment (NHMRC Information Paper 2015). In contrast, the Homeopathy Research Institute (HRI) concluded that homeopathic remedies are biologically effective in spite of their highly diluted state (Witt et al. 2007:128-138). Even though scant scientific evidence supports homeopathic treatments, they are still prevalent worldwide.

In order to build upon the foundations of homeopathy, we aim to unravel its origins during the late nineteenth century, the era of peak popularity and usage. Here we use liquid chromatography-tandem mass spectrometry (LC-MS/MS) techniques to identify the ingredients in one of the most sought after commercially manufactured homeopathic kits. Although the substances utilized by Humphreys in his original products predate the mandated medicine ingredient lists, they can be both identified and quantified through unique product ion m/z transitions (Saji et al. 2011:3951; Rajana et al. 2018:9). Previous studies have successfully utilized similar mass spectrometry techniques to identify unknown ingredients as means of artifact analyses for historical medicines (White 2021). Implementing this science-based framework will provide context for understanding how homeopathy thrived in an era where allopathy had begun to implement scientific change in and improvements to its practices.

Mass Spectrometry Analysis of Humphreys’ Homeopathic Remedies

Materials and Methods

Materials Hydrochloric acid (HCl), formic acid, acetonitrile, and methanol were from Sigma-Aldrich. Apigenin (4’,5,7-Trihydroxyflavon) and sucrose standards were also from Sigma-Aldrich. Humphreys’ Homeopathic Specifics (CDM2003.025.001)

and plant standards (chamomilla, mullein) were provided by the Country Doctor Museum in Bailey, NC. Purified 18 M Ω deionized water was from a Siemens high-purity water system.

Sample Preparation A pill from each remedy analyzed was diluted in an 0.12 M Hydrochloric Acid (HCl). Each corresponding plant-based standard was initially prepped using a mortar and pestle to create a fine powder, if needed, followed by dilution 0.12 M HCl. The solutions were then transferred to Amicon Ultra-0.5 mL 3K filter tubes and centrifuged for 20 min at 30,000 x g. The filtrate was then transferred to LC vials containing 10 μ L inserts.

LC-MS/MS Analysis A SciEx 3200 triple quadrupole LC-MS/MS equipped with a Gemini 3 μ m NX-C18 110 Å LC column (50 x 2 mm) was used. Direct infusion electrospray ionization (ESI) was used to develop two MS detection methods. For both, positive ion mode standard conditions were applied with ESI source settings; electrospray capillary voltage 0.8kV, source temperature 500°C, declustering and entrance potentials of 40V and 10V, respectively.

First, sucrose content in each of the four samples was assayed, and second, mass transitions consistent with apigenin were monitored. For content determination, an analysis was performed in positive ion multiple reaction monitoring (MRM) mode for two mass transitions, characterizing fragments of sodiated sucrose (m/z 365 \rightarrow 203, 365 \rightarrow 185). Specific ESI settings applied were declustering potential of 56V and collision energies of 29V and 27V, respectfully, for each monitored transition. LC solvents were Solvent A: 95:5 DI H₂O and Acetonitrile with 0.1% Formic Acid, and Solvent B: Acetonitrile. Gradient elution took place via 100% A 0-2 mins, ramp to 75% A mins 3-5 and 25% B. The total analysis time was 5 min. The flow rate was 0.200 mL min⁻¹ and the injection volume was 1.00 μ L.

For apigenin, an analysis was again performed in positive ion MRM mode for two mass transitions characterizing fragments of apigenin (m/z 271 \rightarrow 153, 271 \rightarrow 91). Specific ESI settings applied were declustering potential of 51V and collision energies of 47V and 97V, respectfully, for each monitored transition. LC solvents were Solvent A: 95:5 DI H₂O and Acetonitrile with 0.1% Formic Acid, Solvent B: Acetonitrile, and Solvent C: Methanol. Isocratic elution took place via 40% A, 20% B and 40% C for 5 min. The flow rate was 0.200 mL min⁻¹ and the injection volume was 1.00 μ L.

Data Analysis Statistical analysis was performed using Excel for MAC 2019, version 16.24, and SCIEX MultiQuant Software, version 3.0. Raw chromatograms were interpreted using OriginPro 9 software. Figures were modified using Adobe Illustrator CS6.

Results

Our goal was to first determine if Humphreys' marketed treatments contained similar ingredients. Each remedy was advertised to treat a different disorder, as shown in Figs. 4a and 4b (*Images courtesy of the Country Doctor Museum in Bailey, NC*).

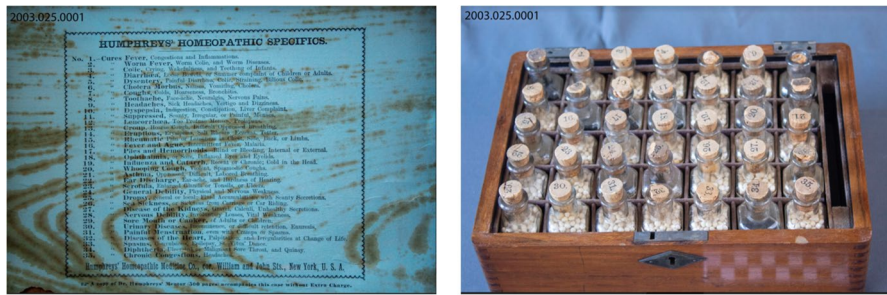


Fig. 4 (a) Humphreys' Homeopathic Specifics table of disorders that accompanied each kit to serve as a directive for the consumer. (b) Humphreys' Homeopathic Specifics kit containing 35 remedies each advertised to treat a different ailment.

It has been previously shown that mass spectrometry is a powerful analytical technique to determine the contents of solutions, specifically medications, through mass-to-charge (m/z) ratios and species-specific transitions (Nizzia et al. 2013:394; Guerra et al. 2015; Croote et al. 2016:16022; Seki et al. 2021:461877; Ouyang et al. 2017:28–286; Baghel et al. 2017; Medina-Pérez et al. 2020:358–367; Wang et al. 2019:815–824; Hamlet 1998:451–465; Cortese et al. 2019). Here, we utilized electrospray ionization (ESI)-triple quadrupole tandem MS to explore base peaks in the four samples to determine if each homeopathic specific contained the same common ingredients. When originally deciding on the samples for our analysis, we specifically chose ones that did not share any likeness in the ailments they treated.

Remedies culled include: #32 Disorders of the Heart, #4 Diarrhea, #17 Piles, and #32 Diphtheria. The spectra showcasing overall m/z (MS1) within the range 50–500 m/z for each sample are shown in Fig. 5a–d.

Figure 5 highlights that the samples are very similar to each other, providing evidence to support that Humphreys' specifics did not have formulae unique to each remedy. Specifically, the spectra show the same base peak ions, or most prevalent ions (m/z 433, 381, 365, 271, 203, 185), in each sample. Essentially these mass spectra showcase the ionized, or charged, components within each sample sorted by m/z , which can be selected and further analyzed to unveil structural information based upon further m/z sorting.

Tandem mass spectrometry (MS/MS) techniques were used to identify the components associated with six of the primary base peaks shared between the four remedies identified using the MS1 scan, as highlighted in Fig. 5. Each base peak served as the primary parent ion when examined via Q1 MS scanning in positive ion mode, followed by product ion scanning while optimizing fragmentation parameters to obtain mass transitions for qualitative determinations of components. Through the utilization of this method, five of the six base peak ions were identified as originating from sucrose, as summarized in Fig. 6 (Beneito-Cambra et al. 2009:907–921; Boutegrabet et al. 2012:13059–67; Hiraoka et al. 2010:2431–38; Patti et al. 2010:121–128; Pradeep et al. 2019:727–736).

As shown in Fig. 6a, the primary base peak ions were attributed to sodiated (m/z 365) and potassiated forms (m/z 381) of sucrose. These forms are commonly

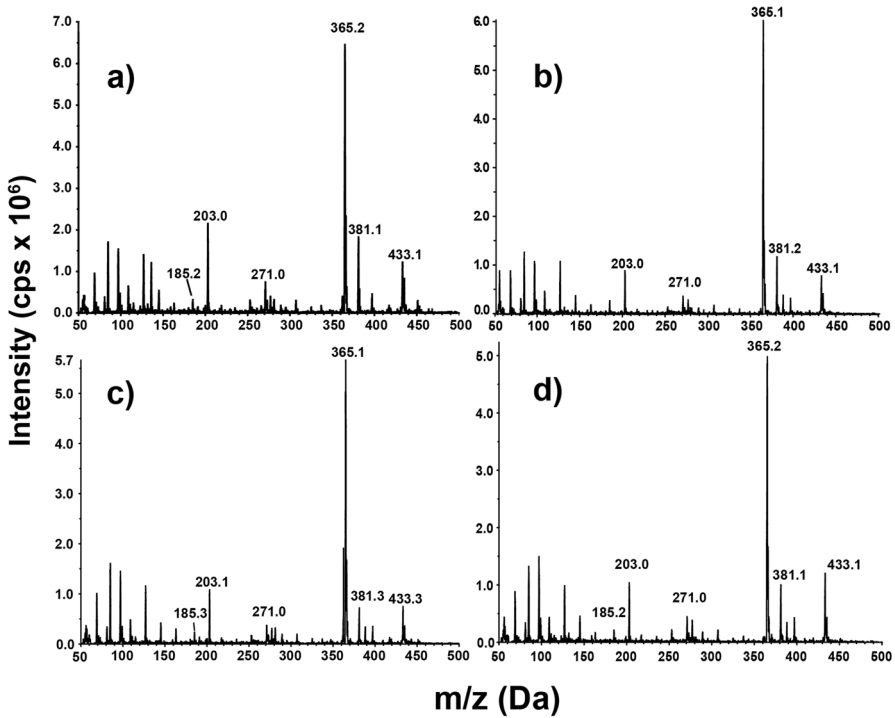


Fig. 5 Mass spectra of Humphreys’ Homeopathic Specifics showcasing the commonality of six primary base peaks unassociated with solvent in samples (a) #32 Disorders of the Heart, (b) Diarrhea, (c) #17 Piles, and (d) Diphtheria. The six primary base peak ions present in a-d were selected and further analyzed using tandem mass spectrometry techniques.

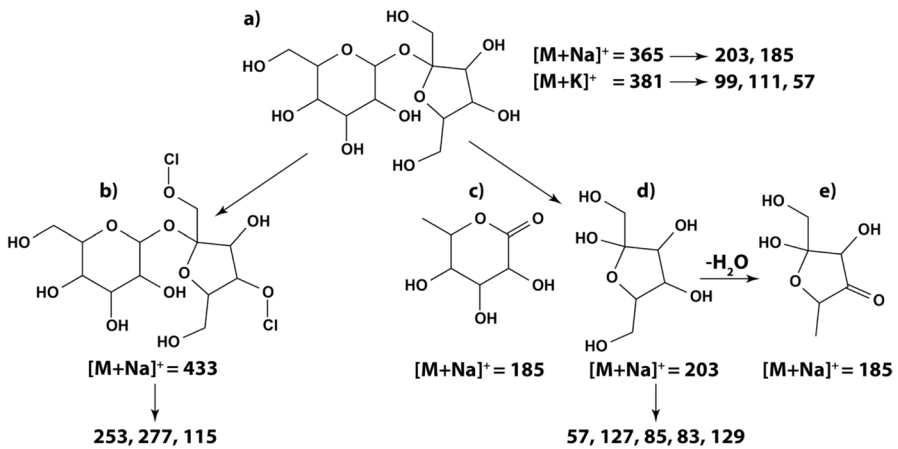


Fig. 6 Mass-to-charge ratios and product ion transitions of five prominent peaks, originating from sucrose-related compounds and identified in all four of Humphreys’ Homeopathic Specifics.

shown in positive ion mode when using ESI-MS, however the interactions specifically with glycosidic bonds in sucrose result in the sodiated form of sucrose being the most stable and most prevalent form (De Leoz et al, 2018:426–38). The larger resultant compound from these two forms was also sodiated, but involved chlorine addition to the external hydroxyl groups, as shown in Fig. 6b. Formation of the chlorinated compounds was introduced as a result of HCl that was used to provide an acidic environment for pill dissolution (Boutegrabet et al. 2012:13059–67).

Sucrose is composed of both fructose and glucose, thus when fragmented using MS/MS or digested in hydrochloric acid, cleavage at the glycosidic linkage can occur resulting in detection of individual sodiated glycosidic ions (Beneito-Cambra et al. 2009:907–921; Patti et al. 2010: 121–128). Each glycosidic ion can be attributed to either sodiated glucose, shown in Fig. 6c, fructose, shown in Fig. 6d, or a dehydrated form of either ion, shown in Fig. 6e. Each species transition shown in Fig. 6 was validated using a sucrose standard equal to the mass of one Humphreys' Homeopathic Specifics pill.

The final base peak ion, m/z 271, was identified using similar methodology. Notably, this ion was the only base peak ion identified that did not have a species transition corresponding to sucrose. The overall product ion spectra comparing the MS/MS for m/z 271 from one of the Humphreys' treatments (#17 Piles), a sucrose standard, and the apigenin standard are shown in Figs. 7a–c, respectively. The resultant m/z 271 \rightarrow 153, 91, 69 species transitions were consistent with apigenin in the assortment of Humphreys' samples, as demonstrated by the match between the sample spectrum in Fig. 7a and the standard shown in Fig. 7c (Solich et al. 2010:1271–80). The isolation and fragmentation of m/z 271 from the sucrose standard (Fig. 7b) showed no similarities to the apigenin standard or Humphreys' sample analysis. The inset of Fig. 7c shows the likely fragmentation locations for the apigenin compound upon MS/MS analysis resulting in the most intense m/z 153 as well as the secondary m/z 91 peaks.

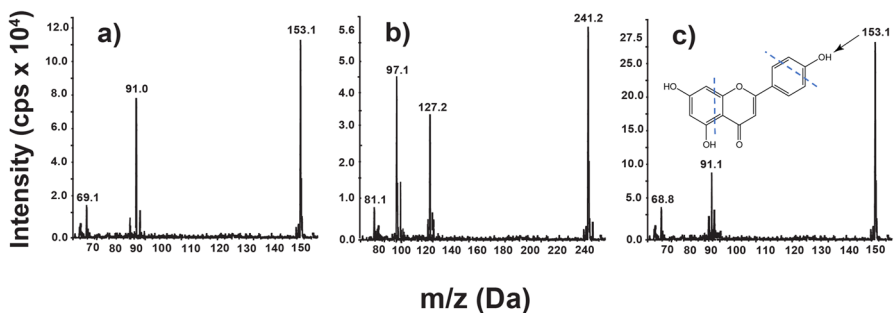


Fig. 7 MS/MS of m/z 271 detailing the determination of m/z in Humphreys' Homeopathic Specifics as apigenin through comparison of (a) #17 Piles, (b) Sucrose standard, and (c) Apigenin standard of equal mass to the homeopathic pill. (c) includes the structure of apigenin with the location of fragmentation corresponding to the base peak ion formed upon MS/MS analysis of apigenin.

Liquid Chromatography-Mass Spectrometry Analysis

In order to quantify both apigenin and sucrose in each of Humphreys' Homeopathic Specifics, LC-MS/MS analyses were performed using positive ion MRM mode for the two most prevalent species transitions of apigenin and sucrose, m/z 271 \rightarrow 153, 91 and m/z 365 \rightarrow 203, 185, respectively. Using linear regression analyses based on both apigenin and sucrose standard responses, the average percent mass of these ingredients was determined for each specific for $n = 3$ pills. These data are summarized in Table 1. Based on our analysis, the overall average mass of sucrose in the pills was determined to be $64.27 \pm 6.25\%$ (g/g), which was two orders of magnitude larger than the average mass percent (g/g) of apigenin, determined to be $0.850 \pm 0.05\%$ (g/g).

The table shows that there is relatively satisfactory interpill precision based on the standard deviation values (RSD of 5 – 13%), but the intrapill precision shows a larger approximate 20% spread. Coupling these data with the aforementioned MS/MS analyses showing two primary ingredients in each sample, we hypothesize that the crude methods of nineteenth-century manufacturing most probably resulted in the low intraspecific precision, as each of the remedies do not show any evidence of being differently manufactured to treat a specific disorder. The better interspecific precision could possibly be a result of when the respective specifics' pills were manufactured in relation to others.

The resulting gap in % (g/g) analysis – i.e., the remaining 40% of pill components - can most likely be attributed to the presence of additional filler compounds, possibly metal-based compounds such as calcium carbonate, that were not shown in initial MS analyses due to instrumental limitations. Future studies involving additional MS techniques will be focused on identifying the make-up of this remaining unknown content (Olesik 1991:12A-21A).

Identification of Potential Apigenin Source

Based on the popularity of flavonoids (i.e., chamomilla), a common source of apigenin, in nineteenth-century alternative treatments and when further coupled with the relative ubiquitous nature of sucrose, apigenin can be considered the active ingredient of Humphrey' samples (Hatfield 2004: 118-119, 191-193, 263-265; Gupta et al. 2010:895-901; Roy et al. 2016:56-68; Chandra et al. 2016). As

Table 1 Content of sucrose and apigenin in analyzed Humphreys' samples.

Sample	Disease	Percent mass ^a	
		Apigenin	Sucrose
#4	Diarrhea	0.789 \pm 0.002	52.24 \pm 5.31
#17	Piles	0.830 \pm 0.002	58.55 \pm 5.67
#32	Heart	0.903 \pm 0.005	71.48 \pm 3.82
#34	Diphtheria	0.876 \pm 0.007	74.65 \pm 10.21

^ag/g \pm s.d. for $n=3$

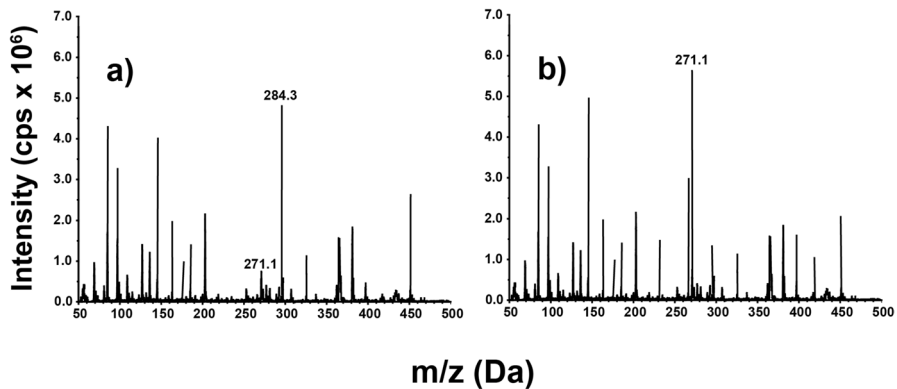


Fig. 8 Mass spectra of sample (a) chamomilla and (b) mullein samples as a comparison to identify the source of apigenin (m/z 271) in Humphreys' remedies.

previously mentioned, flavonoids were largely found in period alternative forms of treatment. We thus considered two widespread sources of apigenin—chamomilla and mullein. Additionally, Humphreys' later listed chamomilla as an ingredient in his remedies following the establishment of the FDA and subsequent labeling mandates in the later twentieth century (National Museum of American History Collections: 1980.0769.57). In order to distinguish between the two selected sources, and to determine the possible vegetable source of apigenin used in Humphreys' remedies, we applied the same MS/MS techniques previously described to analyze both a mullein and a chamomilla sample.

Figure 8 shows that the m/z 271 species was present in both chamomilla in Fig. 8a and mullein in Fig. 8b, respectively. Mullein showed a significantly more intense m/z 271 peak compared to the most intense ion in the chamomilla standard sample. However, the chamomilla sample predominantly exhibited m/z 284, which is inconsistent with apigenin. Comparing to our Humphrey's specifics analysis MS1 spectra, no m/z 284 was seen in appreciable quantity, which points to very little of this detected ion in our samples. Based on the m/z 271 presence similarities between the analyzed pills and mullein, we suggest that the source of apigenin is more likely to be mullein and not chamomilla, a point on which we elaborate below.

Conclusions

Frederick K. Humphreys utilized homeopathic practices to establish his namesake medicinal company. The principles of homeopathy in the United States likewise applied the theory of dilution to patients, only prescribing small amounts of medicine to treat a condition, originating from the pioneer teachings of Samuel Hahnemann (Haller 2015: 9-37). To assess the claims of both homeopathic physicians and patients of the late nineteenth century, MS/MS analysis was used to analyze the ingredients in four separate Humphreys' Homeopathic Specifics.

Sucrose and apigenin were identified as primary components in Humphreys' specifics through the utilization of MS/MS techniques. These findings are further highlighted by Humphreys' article "Homeopathy vs. Allopathy" published in 1847 in *The American Journal of Homeopathy*. Although his primary goal in writing the article was to inform the American public about the success of homeopathy in comparison to traditional medicine, Humphreys (1847:141) did use his own company as an example treatment standard. In doing so, he outlined the preparatory steps used by his company to manufacture the remedies; specifically identifying the use of sugar to prepare the remedies as "not to offend taste," and the harvesting of vegetable medicines during times of bloom (Humphreys 1847: 141). This account establishes that sucrose was added to each remedy solely for the benefits of consumption, not medicinal value, while apigenin would be classified as an active ingredient in terms of Humphreys' vegetable medicine. To account for the incomplete quantitation of each remedy, when combining the detected % (g/g) of both sucrose and apigenin, it is clear that additional filler ingredients were included in the overall mixture of the remedy. Humphreys did list a variety of ingredients in his later remedies, marketed in the early twentieth century following the establishment of the FDA (National Museum of American History Collections: 1980.0769.57). Other metallic-based ingredients popularly used by Native Americans, in which Humphreys' drew inspiration, such as, Calcareo (CaCO_3), were included in his later remedies, but cannot be easily identified using our previously detailed analytical technique (Hatfield 2004:191-193).

Modern comparisons

Although these data do demonstrate that Humphreys' specifics contained the same basic ingredients for varying disorders, apigenin can be linked to widespread medicinal value during Humphreys' time period. Although Humphreys' pills were primarily composed of sucrose, an additive for taste, apigenin was also included as a basis for treatment in all ailments advertised. Dating back to early Native American and European folk medicine, sources of apigenin, primarily flavonoids, were used to treat conditions similar to those found in this study. (Hatfield 2004:190-200). Modern studies have reinforced the use of apigenin in homeopathy, revealing anti-inflammatory, anti-bacterial, and anti-viral qualities (Salehi et al. 2019). Yan, et al. (2017) explored the benefits of apigenin as an anti-cancer therapy, concluding the data showed promise of its use. Of note, modern dosages of apigenin are approximately 5 mg day⁻¹ whereas the dosage of apigenin recommended by Humphreys in his homeopathic treatment guide was approximately 0.016 mg day⁻¹ (6-12 tablets per dose at 4X per day) (Humphreys 1884; Swanson Vitamins). Humphreys did note that patients with more chronic illness could continuously take the specifics until symptoms improved (Humphreys 1884).

To determine the apigenin source used in the samples, we analyzed two plants known for their high concentrations of apigenin — mullein and chamomilla. Although both can be easily grown in the hardiness zones 3-9, which encompass the geographic location of Humphreys' company in New York state, mullein, specifically, has an affinity to grow easily and rapidly, and is often considered to be a nuisance

as a common weed (*Spruce 2021; Dave's Garden 2021*). When coupled with the abundance of m/z 271 in mullein, compared to the decreased intensity of this ion from chamomilla analysis, the possibility certainly exists that Humphreys' sourced his apigenin from common mullein as opposed to the more elegant chamomilla.

Discussion

Humphreys' Homeopathic Medicine Co. occupied a large sector of homeopathic medicine, selling remedies directly to the consumer. However, until the establishment of the FDA, medicine companies like Humphreys' were not mandated nor required to list ingredients on their labels. Therefore, the use of chemical analyses, such as these, can be applied to track the evolution of the alternative form of treatment and as a result, better understand homeopathy at the height of its popularity. The resultant analytical results can then be applied to known historical data, such as patient testimonies, to offer a more comprehensive analysis of the effectiveness and possible validity of these treatments from a scientific standpoint. In future studies, the analytical techniques used in this study can be applied and expanded to further investigate the possibility of other filler ingredients that Humphreys used in his remedies, as well as both Humphreys' competitors and those that marketed their medications solely to homeopathic physicians.

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