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APPLICATION NOTE

Tannic Acid: a Novel Calibrator for Facile and Accurate Mass Measurement of Electrospray Ionization Mass Spectrometry

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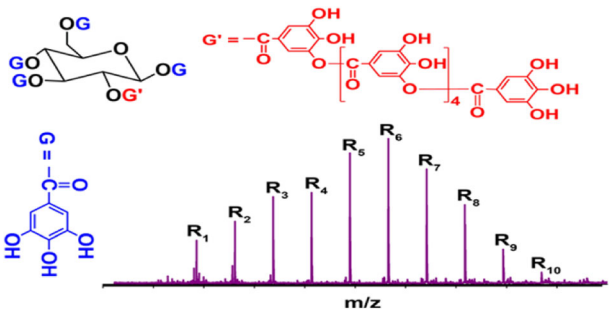

Abstract. Accurate mass calibration is beneficial to the identification of the unknown compounds quickly and accurately. The ESI mass spectrum of tannic acid (TA) tends to a normal distribution of the cluster ion peaks in *m/z* range from 371.0368 to 1739.1169. Based on the interesting result, we reported the use of TA, a natural plant polyphenol, as a novel calibrator for electrospray ionization mass spectrometry (ESI MS), which has the following three advantages, including (1) easy preparation, (2) the calibration range of *m/z* 200~2000, and (3) the calibration error is around 3.00 ppm in positive ion mode, which is less than the use of sodium formate (SF) and Prod #88323 calibrators. This TA calibrator has great potential for the wide applications in biological, chemical, and pharmacal analysis.

Keywords: Tannic acid, Calibrator, Mass measurement, Electrospray ionization mass spectrometry

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Introduction

Accurate mass measurement plays a key role in confidently determining unknown compounds, especially in complex samples [1–4]. To get accurate mass, high-resolution mass spectrometer, sophisticated correction routine, and suitable calibrator are indispensable [2–5]. Internal and external calibration methods are applied to mass calibration of analyte for mass spectrometry (MS). Internal calibration method could gain higher mass accuracy because the analyte and calibrator would share more coincident parameters in analysis than they would with external mass calibration [6, 7]. The most favorite way to carry out internal calibration is to mix calibrator and analyte together and then simultaneously ionize the mixture. However, due to the different natures of calibrator and analyte, the ionization suppression effect would occur among them. Li et al. introduced dual nano-sprayers, one of which contains the calibrator and the other the analyte, to effectively reduce such kind of suppression and successfully determine the targeted compound in urine—creatinine, a common metabolin of human muscle with the mean mass errors of 3.1 ppm [5]. However, two metal tubes, two nano-sprayers, and an alternating current power supply are needed in this internal calibration method. Due to its easy operation and little interference, the external calibration method is used more broadly in electrospray ionization mass spectrometry (ESI MS) analysis than that of the internal calibration method.

In the external calibration method, the solutions of sodium formate (SF), sodium trifluoroacetate, polyethylene glycol, polypropylene glycol solution, and ionic liquid were reported as calibrators for mass spectrometer [6–8]. For example, the solution of isopropanol/de-ionized water (50:50, *v*/*v*) with 0.2% formic acid (*v*/*v*) was prepared as the solvent of SF calibrator. Then, dissolve 1 mL of 1.0 mol/L NaOH solution into 99 mL of the above solvent to prepare 1.0×10^{-2} mol/L SF calibrator. The use of high-concentration NaOH solution as calibrator may damage mass spectrometer detector, even shorten the life of the instrument for a long period. A polypropylene glycol solution of 5 μg/mL was prepared by diluting the 5 mg/mL stock solution in 2-propanol/water (70:30, *v*/*v*) that contained an overall concentration of 0.5 mmol/L ammonium acetate [6]. Though polypropylene glycol solution was used as calibrator for Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) in the low concentration, the calibration range is from *m/z* 700 to 1200. Using 1-butyl-3-methylimidazolium tricyanomethide as ionic liquid calibrator, it delivers positive cluster ions suitable for mass calibration in

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the *m/z* 100–4000 range and covers the *m/z* 100–2000 range in negative-ion direct analyses in real time (DART) FT-ICR MS [8]. However, in ESI mode, the entrance section of the transfer capillary is heated to about 200 °C by virtue of the hot desolvation gas, which leads to the ionic liquid calibrator broken down. Hence, it is crucial to choose stable, mild solution with low concentration, and wide *m/z* range as calibrator to prolong the life of ESI mass spectrometer.

Tannic acid (TA), a kind of polyphenols, contains numerous phenolic hydroxyl groups in the chemical structure, which make it unique physical chemistry properties, excellent biological and pharmacological activities as antioxidation, anti-inflammatory function, antimicrobial action, and cardiovascular protection.

Mass spectrometry (MS) has become a powerful technique and a routine analytical method in modern analytics and drug research [9–11]. In this paper, TA was analyzed by ESI MS because of its features of high sensitivity, simple sample preparation, low sample consumption, and gentle ionization method [12]. Due to the numerous phenolic hydroxyl groups in TA, the positive mode mass spectrum of TA shows a normal distribution of the cluster ion peaks in *m/z* range from 371.0368 to 1739.1169. The interesting result makes TA an excellent calibrator to detect six samples including N-benzyl-hexadecanamide (NBHD), N-benzyl-(9Z, 12Z, 15Z)-octadecatrienamide (NBOT), N-benzyl-(9Z, 12Z)-octadecadienamide (NBOD), N-(3-methoxybenzyl)-(9Z, 12Z)-octadecadienamide (NMOT), β-cyclodextrin (β-CD), and mono-(6-diethylenetriamine)-6-deoxy)-β-cyclodextrin (M-β-CD).

Experimental

Materials

NaOH was provided by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). TA, β-CD, methanol, isopropanol, and formic acid were purchased from Sigma-Aldrich (MA, USA). NBHD, NBOT, NBOD, and NMOT were obtained from Wuhan Huashite Industrial Biotechnology Development Co., Ltd. (Wuhan, China). M-β-CD was from Tocopharm Co., Ltd. (Shanghai, China). Pierce™ LTQ Velos ESI positive ion calibration solution (Prod #88323) was gained from Thermo Fisher Scientific (MA, USA). The de-ionized water was provided by a Milli-Q system (Millipore, Billerica, MA).

TA solution of 1.0×10^{-2} mmol/L was prepared by diluting the 1 mmol/L stock solution in formic acid/methanol solution (0.1:100, *v/v*). Solutions of NBHD, NBOT, NBOD, NMOT, β-CD, and M-β-CD were prepared in formic acid/methanol solution (0.1:100, *v/v*) with concentrations of 1.0×10^{-2} mmol/L for micrOTOF II time-of-flight MS analysis.

Solutions of NBHD, NBOT, NBOD, and NMOT were prepared in methanol solution with concentrations of 1.0×10^{-2} mmol/L for LTQ ORBITRAP XL MS analysis. Solutions of β-CD and M-β-CD were prepared in de-ionized water with concentrations of 1.0×10^{-2} mmol/L for LTQ ORBITRAP XL MS analysis.

Mass Spectrometry

Mass spectra were gained using either a micrOTOF II time-of-flight mass spectrometer (Bruker Daltonics, Germany) or LTQ ORBITRAP XL mass spectrometer (Thermo Fisher Scientific, Germany) equipped with electrospray ionization source. The experimental parameters of micrOTOF II time-of-flight mass spectrometer were as follows: flow rate of the sample solution, 3 μL/min; flow rate of nitrogen, 4 L/min; temperature of dry gas, 200 °C; voltage of capillary, 2.6 kV; voltage off end plate offset, 0.5 kV. MicrOTOF control software of the ESI MS instrument was used to record the full scan mass spectra at the *m/z* range of 50~2000. For the LTQ ORBITRAP XL, a spray voltage of 2.50 kV, a tube lens voltage of 230 V, an in-source fragmentation of 5.0 eV, and a *m/z* range of 2000 to 4000 were selected.

Results and Discussion

Mass Calibration on the micrOTOF II Time-of-Flight Mass Spectrometer

Figure 1a shows the chemical structure of TA. There are numerous phenolic hydroxyl groups in the chemical structure of TA.

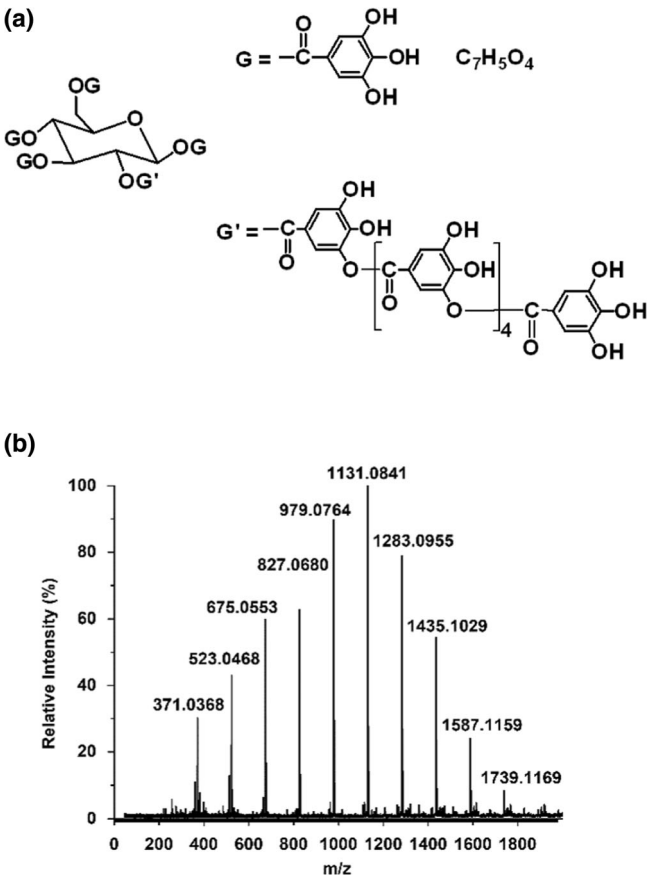

Figure 1. Chemical structure of TA (**a**) and positive ion ESI mass spectrum of 1.0×10^{-2} mmol/L TA in 0.1% formic acid/methanol solution (*v/v*) (**b**)

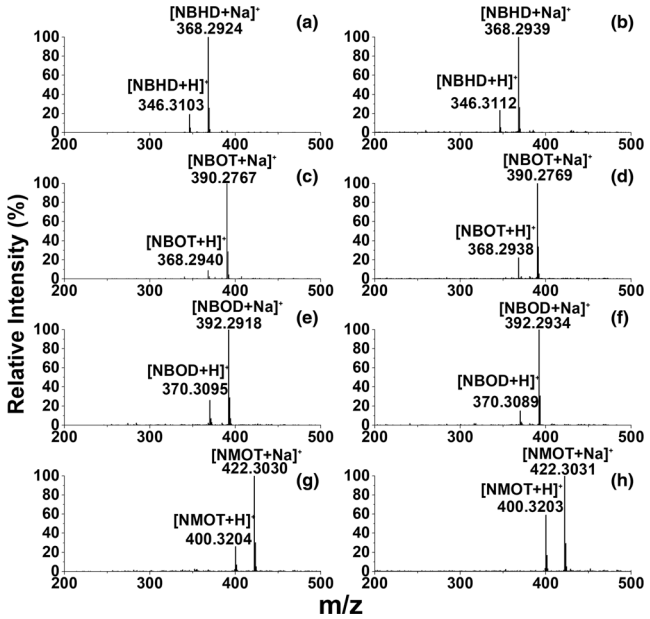

Figure 2. Positive ion ESI mass spectra of amides: (**a**) NBHD calibrated by TA, (**b**) NBHD by SF, (**c**) NBOT by TA, (**d**) NBOT by SF, (**e**) NBOD by TA, (**f**) NBOD by SF, (**g**) NMOT by TA, and (**h**) NMOT by SF, respectively. The amides were dissolved in 0.1% formic acid/methanol solution (*v/v*) at a concentration of 1.0×10^{-2} mmol/L

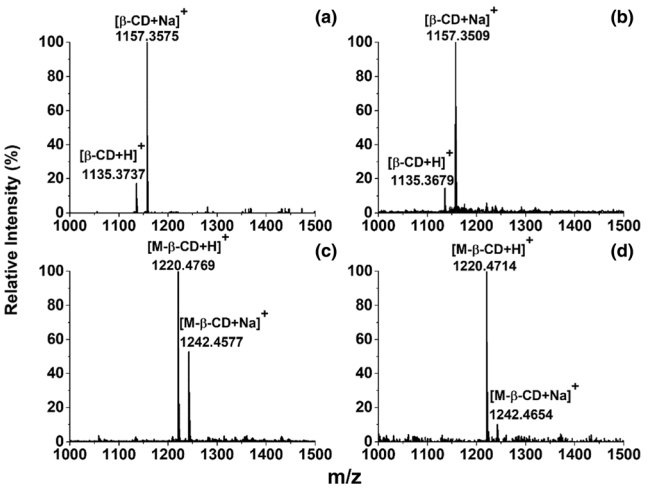

Figure 3. Positive ion ESI mass spectra of cyclodextrins: (**a**) β-CD calibrated by TA, (**b**) β-CD by SF, (**c**) M-β-CD by TA, and (**d**) M-β-CD by SF, respectively. The cyclodextrins were dissolved in 0.1% formic acid/methanol solution (*v/v*) at a concentration of 1.0×10^{-2} mmol/L.

Figure 1b shows the positive ion ESI mass spectrum of 1.0×10^{-2} mmol/L TA in 0.1% formic acid/methanol solution (*v/v*). Ten strong peaks corresponding to the potassium adduct ions of TA, $[glucose+C_7H_5O_4\text{-}H+K]^+$, $[glucose+2C_7H_5O_4\text{-}2H+K]^+$, $[glucose+3C_7H_5O_4\text{-}3H+K]^+$, $[glucose+4C_7H_5O_4\text{-}4H+K]^+$, $[glucose+5C_7H_5O_4\text{-}5H+K]^+$, $[glucose+6C_7H_5O_4\text{-}6H+K]^+$, $[glucose+7C_7H_5O_4\text{-}7H+K]^+$, $[glucose+8C_7H_5O_4\text{-}8H+K]^+$, $[glucose+9C_7H_5O_4\text{-}9H+K]^+$, and $[glucose+10C_7H_5O_4\text{-}10H+K]^+$, appear at *m/z* 371.0368, 523.0468, 675.0553, 827.0680, 979.0764, 1131.0841, 1283.0955, 1435.1029, 1587.1159, and 1739.1169, respectively. The reason may be that these phenolic hydroxyl groups attached to the core structure could make TA easily form the ionized molecules in electrospray ionization. Due to its accurate cluster ion pattern with normal distribution in the *m/z* range

Table 1. Accurate Mass Measurement Results for the Six Compounds

Sample	Detected ion	Exact mass of the ion	Calculated mass of the ion	Relative error (ppm)
NBHD	$[M+H]^+$	346.3104	(a) 346.3103	(a) − 0.29
			(b) 346.3112	(b) 2.31
	$[M+Na]^+$	368.2924	(a) 368.2924	(a) 0.00
			(b) 368.2939	(b) 4.07
NBOT	$[M+H]^+$	368.2948	(a) 368.2940	(a) − 2.17
			(b) 368.2938	(b) − 2.72
	$[M+Na]^+$	390.2767	(a) 390.2767	(a) 0.00
			(b) 390.2769	(b) 0.51
NBOD	$[M+H]^+$	370.3104	(a) 370.3095	(a) − 2.43
			(b) 370.3089	(b) − 4.05
	$[M+Na]^+$	392.2924	(a) 392.2918	(a) − 1.53
			(b) 392.2934	(b) 2.55
NMOT	$[M+H]^+$	400.3210	(a) 400.3204	(a) − 1.50
			(b) 400.3203	(b) −1.75
	$[M+Na]^+$	422.3030	(a) 422.3030	(a) 0.00
			(b) 422.3031	(b) 0.24
β-CD	$[M+H]^+$	1135.3770	(a) 1135.3737	(a) − 2.91
			(b) 1135.3679	(b) − 4.32
	$[M+Na]^+$	1157.3590	(a) 1157.3575	(a) − 1.30
			(b) 1157.3509	(b) − 4.41
M-β-CD	$[M+H]^+$	1220.4774	(a) 1220.4769	(a) − 0.41
			(b) 1220.4714	(b) − 4.92
	$[M+Na]^+$	1242.4594	(a) 1242.4577	(a) − 1.37
			(b) 1242.4654	(b) 4.83

Each compound was calibrated by TA (indicated as (a) and SF (indicated as (b)

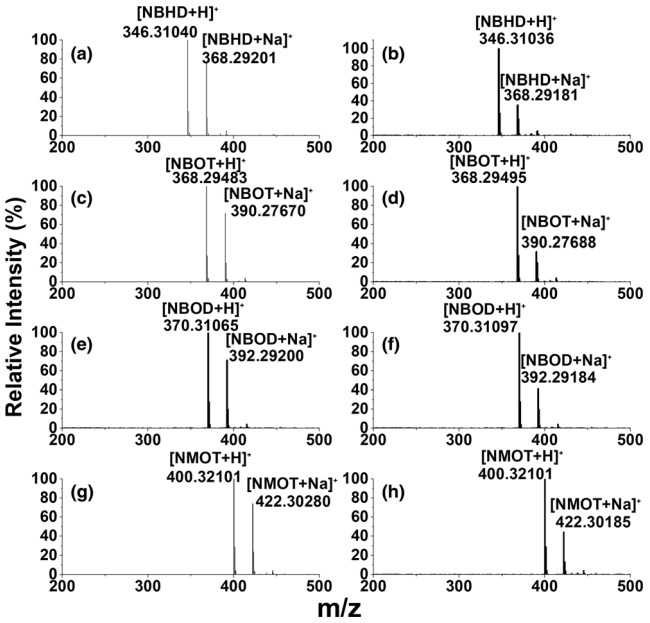

Figure 4. Positive ion mass spectra of amides: (**a**) NBHD calibrated by TA, (**b**) NBHD by Prod #88323, (**c**) NBOT by TA, (**d**) NBOT by Prod #88323, (**e**) NBOD by TA, (**f**) NBOD by Prod #88323, (**g**) NMOT by TA, and (**h**) NMOT by Prod #88323, respectively. The amides were dissolved in methanol solution at a concentration of 1.0×10^{-2} mmol/L

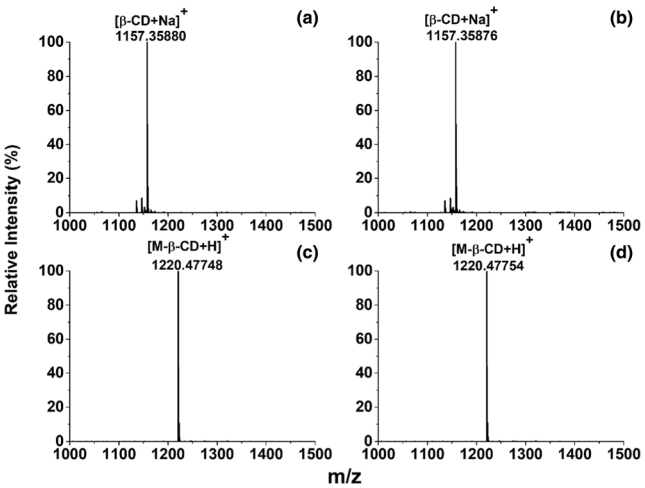

Figure 5. Positive ion mass spectra of cyclodextrins: (**a**) β-CD calibrated by TA, (**b**) β-CD by Prod #88323, (**c**) M-β-CD by TA, and (**d**) M-β-CD by Prod #88323, respectively. The cyclodextrins were dissolved in de-ionized water at a concentration of 1.0×10^{-2} mmol/L

from 300 to1800, TA may be utilized as a reference reagent to calibrate mass of analyte by ESI MS in the positive ion mode.

For comparison, we show the positive ion ESI mass spectra of amides (MW < 1000) in formic acid/methanol solution with TA and SF as calibrators, respectively. In Figure 2, although protonated ions and sodiated adduct ions of samples are detected, there are still some deviations of *m/z* for the same ions in the results between the two calibrators. Taking the analysis of NBOT as an example, the peaks corresponding to $[NBOT+Na]^+$ appear at *m/z* 390.2767 and 390.2769 with the two calibrators, respectively. Table 1 shows the analytical result of the relative error of *m/z* of ions calibrated by the two calibrators. The mass error of $[NBOT+Na]^+$ calibrated by TA is 0.00, but that by SF is 0.51 ppm.

We use β-CD and M-β-CD (MW > 1000) as analytes to further discuss the accurate mass measurement of TA calibrator than that of SF. Similarly, signals of protonated ions and sodiated adduct ions appear in Figure 3; however, there are still some deviations of *m/z* for the same ions in the result with the two calibrators. For example, the peaks corresponding to $[M-β-CD+H]^+$ appear at *m/z* 1220.4769 and 1220.4714 with TA and SF as calibrators, respectively. In Table 1, the relative error of $[M-β-CD+H]^+$ calibrated by TA is − 0.41 ppm, but that by SF is − 4.92 ppm. In conclusion, the result shows that the

Table 2. Accurate Mass Measurement Results for the Six Compounds

Sample	Detected ion	Exact mass of the ion	Calculated mass of the ion	Relative error (ppm)
NBHD	$[M+H]^+$	346.31045	(a) 346.31040	(a) − 0.14
			(b) 346.31036	(b) − 0.26
	$[M+Na]^+$	368.29239	(a) 368.29201	(a) − 1.03
			(b) 368.29181	(b) − 1.57
NBOT	$[M+H]^+$	368.29480	(a) 368.29483	(a) 0.08
			(b) 368.29495	(b) 0.41
	$[M+Na]^+$	390.27674	(a) 390.27670	(a) − 0.10
			(b) 390.27688	(b) 0.36
NBOD	$[M+H]^+$	370.31045	(a) 370.31065	(a) 0.54
			(b) 370.31097	(b) 1.40
	$[M+Na]^+$	392.29239	(a) 392.29200	(a) − 0.99
			(b) 392.29184	(b) − 1.40
NMOT	$[M+H]^+$	400.32101	(a) 400.32101	(a) 0.00
			(b) 400.32101	(b) 0.00
	$[M+Na]^+$	422.30296	(a) 422.30280	(a) − 0.38
			(b) 422.30185	(b) − 2.63
β-CD	$[M+Na]^+$	1157.35899	(a) 1157.35880	(a) − 0.16
			(b) 1157.35876	(b) − 0.20
M-β-CD	$[M+H]^+$	1220.47743	(a) 1220.47748	(a) 0.04
			(b) 1220.47754	(b) 0.09

Each compound was calibrated by TA (indicated as (a) and Prod #88323 (indicated as (b)

relative errors of adducts calibrated by TA is no more than 3.00 ppm, which is less than the use of SF calibrator.

Mass Calibration on the LTQ ORBITRAP XL Mass Spectrometer

To evaluate TA's performance as a calibrator, we conducted the experiments on LTQ ORBITRAP XL mass spectrometer, a type of high-resolution mass spectrometer. We show the positive ion ESI mass spectra of amides in formic acid/methanol solution with TA and Prod #88323 as calibrators, respectively. In Figure 4, although protonated ions and sodiated adduct ions of amides are detected, there are still some deviations of *m/z* for the same ions in the results between the two calibrators. Table 2 shows the analytical result of the relative error of *m/z* of ions calibrated by the two calibrators. Taking the analysis of NBOD as an example, the peaks corresponding to $[NBOD+H]^+$ appear at *m/z* 370.31065 and 370.31097 with the two calibrators, respectively. The mass error of $[NBOD+H]^+$ calibrated by TA is 0.54 ppm, but that by Prod #88323 is 1.40 ppm.

We also use β-CD and M-β-CD as analytes to further discuss the accurate mass measurement of TA calibrator than that of Prod #88323. Signals of $[\beta\text{-CD}+Na]^+$ and $[M\text{-}\beta\text{-CD}+H]^+$ appear in Figure 5. There are still some deviations of *m/z* for the same ions in the result with the two calibrators. For example, the peaks corresponding to $[M\text{-}\beta\text{-CD}+H]^+$ appear at *m/z* 1220.47748 and 1220.47754 with the two calibrators, respectively. The relative error of $[M\text{-}\beta\text{-CD}+H]^+$ calibrated by TA is 0.04 ppm, but that by Prod #88323 is 0.09 ppm. In conclusion, the result shows that the relative error of adducts calibrated by TA is no more than 1.03 ppm, which is less than the use of Prod #88323 calibrator.

All are proved that TA solution can calibrate mass of analyte with lower mass deviation by ESI MS in positive ion mode.

Conclusions

Due to the numerous phenolic hydroxyl groups attached to the core structure, the positive mode ESI mass spectrum of TA shows a normal distribution of the cluster ion peaks in *m/z* range from 371.0368 to 1739.1169. Based on the interesting result, TA was used as a reference reagent to calibrate mass of the six samples including NBHD, NBOT, NBOD, NMOT, β-CD, and M-β-CD by ESI MS in positive ion mode. The result shows that the calibration error of adducts calibrated by TA is no more than 3.00 ppm, which is less than the use of SF and Prod #88323 calibrators. This TA calibrator has great potential for the broad applications in biological, chemical, and pharmacal analysis. The reason that the peak distribution profile changed with the levels of potassium ions is currently in progress in our laboratories.

Acknowledgements

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