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Liquid Chromatography−Tandem Mass Spectrometric Ion-Switching Determination of Chlorantraniliprole and Flubendiamide in Fruits and Vegetables

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The anthranilic and phthalic diamides, chlorantraniliprole (CAP) and flubendiamide (FLU), respectively, represent a new class of very effective insecticides that activate the ryanodine-sensitive intracellular calcium release channel (ryanodine receptor). This paper reports an analytical method for the simultaneous determination of the two insecticides on fruits and vegetables by liquid chromatography−electrospray tandem mass spectrometry operated in the positive and negative ionization switching mode. The two diamides were extracted with acetonitrile and separated on a Zorbax Column Eclipse XDB C8 (4.6 mm × 150 mm i.d., 3 μm) by isocratic elution with a mobile phase consisting of acetonitrile and water with 0.1% formic acid pumped at a flow rate of 0.4 mL/min. The diamides were selectively detected by multiple reaction monitoring for transitions of proton adduct precursor ions simultaneously: positive *m*/*z* 484.3→285 for CAP, *m*/*z* 445.5→169 for internal standard, and negative *m*/*z* 681.4→253 for FLU. For CAP calibration in the positive mode was linear over a working range of 2 to 1000 μg/L with $r > 0.992$. The limit of detection (LOD) and limit of quantification (LOQ) for CAP were 0.8 and 1.6 μg/kg, respectively. For FLU in the negative mode the corresponding values were 1−1000 μg/L for linear working range, with $r > 0.996$ and 0.4 and 0.8 μg/L for LOD and LOQ, respectively. Moreover, the presence of interfering compounds in the fruit and vegetable extracts was found to be minimal. Due to the linear behavior of the MS detector response for the two analytes, it was concluded that the multiple reaction transitions of molecular ions in the ion-switching mode can be used for analytical purposes, that is, for identification and quantification of diamides in fruit and vegetable extracts at trace levels.

KEYWORDS: Chlorantraniliprole; flubendiamide; ion-switching; LC-ESI-MS/MS; residue; vegetables

INTRODUCTION

Chlorantraniliprole (CAP) and flubendiamide (FLU) (**Figure 1**) are new insecticides belonging to the anthranilic and phthalic diamide class sharing a new mode of action on the insect ryanodine receptor (*1*−*6*). CAP is being developed by DuPont Crop Protection and Syngenta Crop Protection, whereas FLU is being codeveloped by Nihon Nohyaku and Bayer CropScience. They both act as allosteric activators on the intracellular sarcoplasmic Ca^{2+} release channel, namely, the ryanodine receptor, and are extremely potent against lepidopterous pests including those resistant to neonicotinoid and pyrethroid insecticides. Pending target crops for these insecticides are cotton, corn, grape, pome fruits, potatoes, and strawberry (*7*). Analysis of FLU in different food matrices was recently reported by HPLC-DAD (*8*) and LC-MS/MS with electrospray ionization (ESI) (*9*) with a limit of quantification (LOQ) in each case of 10 μg/kg. Atmospheric pressure chemical ionization (APCI) is another widely accepted technique for the analysis of pesticide residues at micrograms per kilogram levels in both vegetables and fruits, but it did not prove useful in the present study. The ESI positive and negative ion-switching mode (*10, 11*) in a single run allows simultaneous determination of compounds that give $[M + H]^+$ and $[M - H]^-$ ions or other adducts. The aim of this work is to develop and validate a LC tandem MS ion-switching method for the unambiguous determination of CAP and FLU in a single run in fruits and vegetables.

EXPERIMENTAL PROCEDURES

Chemicals and Standards. Analytical standards of 3-bromo-*N*-[4-chloro-1-(3-chloro-2-methyl-6-(methylcarbamoyl)phenyl]-1-(3-chloro-

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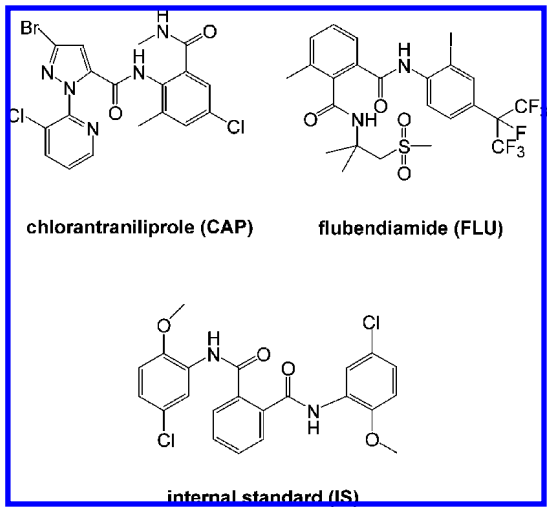

Figure 1. Chemical structures of chlorantraniliprole, flubendiamide, and the internal standard.

pyridin-2-yl)-1,4-pyrazole-5-carboxanilide (CAP) and 3-iodo-*N*′-(2-mesyl-1,1-dimethylethyl)-*N*-{4-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]-*o*-tolyl]}phthalamide (FLU) were provided by the Berkeley Laboratory. The internal standard (IS) *N*,*N*′-bis-(5-chloro-2-methoxyphenyl)phthalamide was from Sigma Aldrich (Steinheim, Germany). Other chemicals were as follows: acetonitrile of HPLC grade (Baker, Milan, Italy); formic acid, sodium chloride, and anhydrous magnesium sulfate from Sigma Aldrich (Milan, Italy); water distilled and filtered through a Milli-Q apparatus (Millipore, Milan, Italy).

HPLC-ESI-MS/MS Analysis. A Varian 1200 L triple-quadrupole tandem mass spectrometer (Palo Alto, CA) coupled with a ProStar 410 autosampler and two ProStar 210 pumps and a 1200 L triple-quadrupole mass spectrometer was used with an ESI source. The Varian MS workstation version 6.7 software was used for data acquisition and processing. Chromatographic separation was performed on a Zorbax Column Eclipse XDB C8 (4.6 mm × 150 mm i.d., 3 μm) (Milford, MA). The mobile phase consisted of (A) acetonitrile 80% (v/v) and (B) double distilled water 20% (v/v) containing 0.1% formic acid. The mobile phase, previously degassed with high-purity helium, was pumped at a flow rate of 0.4 mL/min, and the injection volume was 10 μL. ESI was operated in the positive and negative ion mode. The electrospray capillary potential was set to 65 V, the needle at 5850 V, and the shield at 750 V. Nitrogen at 48 mTorr and 375 °C was used as a drying gas for solvent evaporation. APCI was operated in the positive mode. The capillary potential was set to 65 V, the APCI torch at 450 °C, and the shield at 750 V. Nitrogen at 48 mTorr was set at 400 °C. Full-scan spectra were obtained in the ranges of 250−800 amu for CAP and 400−950 amu for FLU, scan time of 0.75 amu, scan width of 0.70 amu, and detector at 1450 V. For both ESI and APCI the atmospheric pressure ionization (API) housing was kept at 50 °C. Parent compounds were subjected to collision-induced dissociation using argon at 3.80 mTorr in the multiple reaction monitoring (MRM) positive and negative mode. **Table 1** reports the observed mass transitions and collision energy used for quantitation of different diamides. The scan time was 1 s, and the detector multiplier voltage was set to 1450 V, with an isolation width of *m*/*z* 1.2 for quadrupole 1 and *m*/*z* 2.0 for quadrupole 3.

Standard and Working Solutions. Standard stock solutions of CAP and FLU (1000 mg/L) were prepared in acetonitrile by weighing 10 mg of the pure analyte into a 10 mL volumetric flask and diluting to volume. For the MS/MS analysis an intermediary mixed standard solution was prepared daily by diluting the stock solutions with the mobile phase as listed above. Working solutions were prepared by diluting the mixed standard solution with the extract obtained from the untreated fruit and vegetable matrices. All standard solutions were stored in the dark at −20 °C until use.

Efficiency. *Standard Curves and Linearity.* A six-point standard curve was prepared for each compound. Standard solutions were made in triplicate containing the two diamides at 2, 10, 50, 100, 500, and 1000 μg/L. Calibration curves were created by plotting the concentration of each compound against the ratio of the standard peak area/IS area of the monitored transitions. Simple linear regression analysis was performed to calculate the slope and intercept. The correlation coefficient (*r*) for CAP and FLU was also determined.

Repeatability. To evaluate precision, the repeatability was determined of both the instrument and the proposed analytical procedure. Between-day repeatability was calculated by performing six injections of the same standard at 10, 50, 100, 500, and 1000 μg/L for six consecutive days.

Extraction of Diamides from Fruits and Vegetables. Fresh and uncontaminated fruits (apples, grapes, and pears) and vegetables (eggplants, peppers, and tomatoes) were purchased at local markets in Cagliari, Italy. Samples were analyzed, unwashed, and in a raw state. Samples of fruits or vegetables were placed in a blender/cutter (Malavasi, Bologna, Italy) and chopped for 30 s. A portion (5 g) of well-homogenized sample was weighed in a 40 mL screw-capped glass tube, and then 2 g of sodium chloride, 1 g of anhydrous magnesium sulfate, and 20 mL of acetonitrile were added. The tube was agitated for 15 min in a rotary shaker at 9 rpm (FALC Instrumentals, Bergamo, Italy) at room temperature, and 1 mL of the mixture was evaporated to dryness under a gentle nitrogen stream. The residue was dissolved with 200 μL of the mobile phase and submitted to chromatographic analysis in the MRM mode. The amount of sample equivalent in the final extract was 1.25 g/mL.

Recovery Experiment. For recovery assays, a 50 μL aliquot of diamide solution at the desired standard concentration was added to each 5 g sample of fruits and vegetables. The fortification levels were 1.6, 8, 16, 80, and 160 μg/kg. Samples were allowed to settle for 30 min prior to using the extract and were processed later according to the above extraction procedure. Four replicates for each level were analyzed by LC-MS/MS.

RESULTS AND DISCUSSION

Multiple Reaction Transitions. Electrospray (ESI) and atmospheric pressure chemical ionization (APCI) were tested for determination of CAP and FLU in both the positive and negative mode, respectively. ESI in the negative mode did not give any signal for CAP, whereas in the APCI negative mode neither compound was detected with infusion at the rate of 0.6 mL/h of standard solutions of diamides at 1000 μg/L. Thus, ESI in the ion-switching (positive for CAP and negative for FLU simultaneously) mode was chosen for the identification, quantification, and confirmation of insecticides with the most intense response compared with APCI. The full-scan spectrum for CAP (**Figure 2a**) shows the isotopic pattern typical for a structure containing two chlorines and one bromine. The most intense ions were the isotope A + 2 proton adduct (*m*/*z* 484.4) and its corresponding sodium adduct (*m*/*z* 507.6). On the other hand, the FLU spectrum (**Figure 2b**) reports the $[M - H]^-$ and the $[M + {}^{35}Cl]^-$ at *m*/*z* 717.3 and $[M + {}^{37}Cl]^-$ at *m*/*z* 719. The electrospray capillary potential as well as shield and needle voltage was optimized for each compound. Mass fragments of the precursor ions were produced by collision-induced dissociation (CID) using argon at 3.80 mTorr. The collision energy was optimized to achieve the highest sensitivity. CID resulted in the formation of ions unique to each compound. As seen in **Table 1**, a product ion scan of the individual compounds gave unique mass fragments for each insecticide. For CAP we observed intense fragmentation ions at *m*/*z* 285, 453, and 287. For FLU we observed fragments at *m*/*z* 253, 274, and 271. The most intense transitions were chosen to provide selective detection of diamides in fruits and vegetables without the need of a further purification step. Selected reaction monitoring of the precursor−product ion transitions were *m*/*z* 484.3 → 285 for CAP, *m*/*z* 681.4 → 253 for FLU, and *m*/*z* 445.5 → 169 for the IS. The second and third transitions were used for confirma-

Table 1. Analyte ESI-MS/MS (±) Transitions and Instrument Conditions

			first transition		second transition		third transition	
compd	MW	precursor mass (*m*/*z*)	mass (*m*/*z*)	CE (V)	mass (*m*/*z*)	CE (V)	mass (*m*/*z*)	CE (V)
CAP	481	484 $[A + 2]^+$	484.3 → 285	−14	484.3 → 453	−16	484.3 → 287	−14
FLU	682	681 $[M - H]^-$	681.4 → 253	+28	681.4 → 274	+16	681.4 → 271	+16
IS	444	445 $[M + H]^+$	445.5 → 169	−20	445.5 → 171	−28	445.5 → 258	−14

tory purposes for real fruit and vegetable samples. For the HPLC-ESI-MS/MS quantitation of diamides an IS method was used with quantitative determination of peak areas obtained from the MRM of insecticides.

Method Development. Chromatographic separation of the two diamides was achieved with isocratic elution in the reverse phase mode, giving retention times for CAP, FLU, and IS of 4.71, 5.79, and 6.58 min, respectively. For CAP and FLU the calibration was linear over working ranges of 2−1000 and

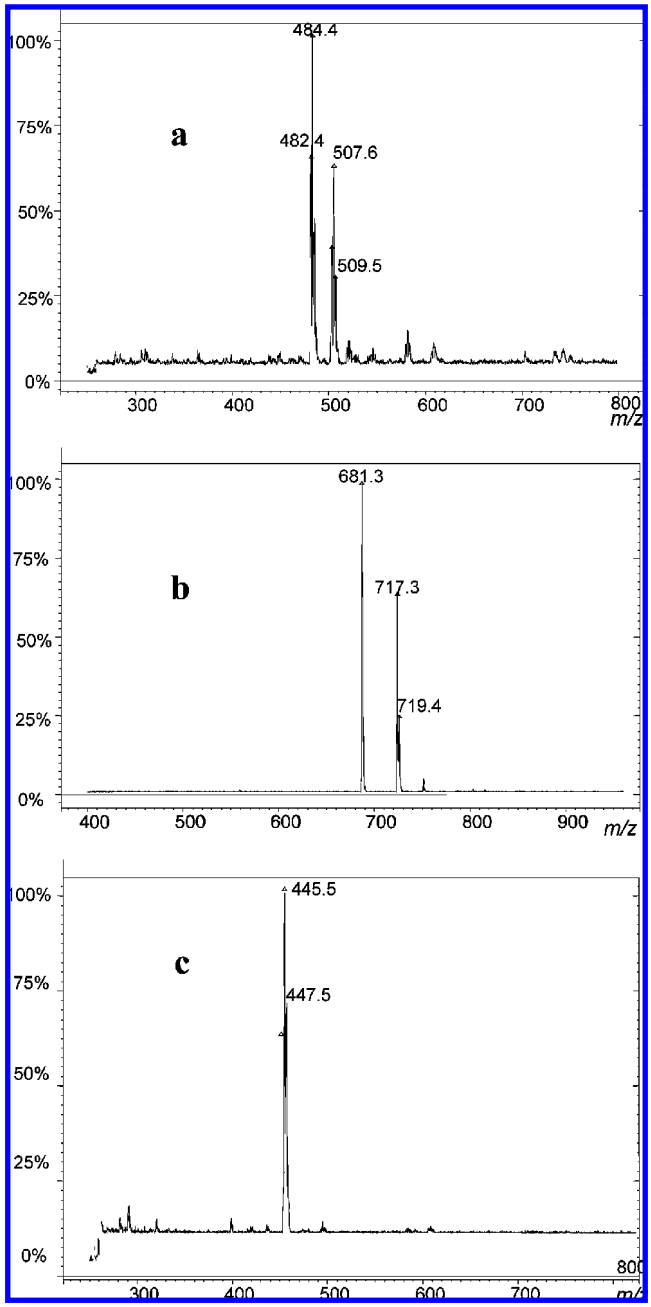

Figure 2. Ion electrospray full scan mass spectra of (**a**) chlorantraniliprole, (**b**) flubendiamide, and (**c**) internal standard.

Table 2. Limit of Detection (LOD) and Quantification (LOQ) (Micrograms per Kilogram) of Chlorantraniliprole, Flubendiamide, and Internal Standard

	CAP				FLU			
matrix	LOD	LOQ	slope	slope ratio	LOD	LOQ	slope	slope ratio
solvent[a]	0.1	0.5	0.202		0.1	0.5	0.200	
eggplant	0.8	1.6	0.011	0.05	0.4	0.8	0.334	1.67
tomato	0.8	1.6	0.022	0.11	0.4	0.8	0.318	1.59
pepper	0.8	1.6	0.022	0.11	0.4	0.8	0.045	0.23
apple	0.8	1.6	0.012	0.06	0.4	0.8	0.217	1.09
pear	0.8	1.6	0.021	0.10	0.4	0.8	0.256	1.28
grape	0.8	1.6	0.016	0.08	0.4	0.8	0.041	0.21

[a] LOD and LOQ of solvent are expressed as μg/L.

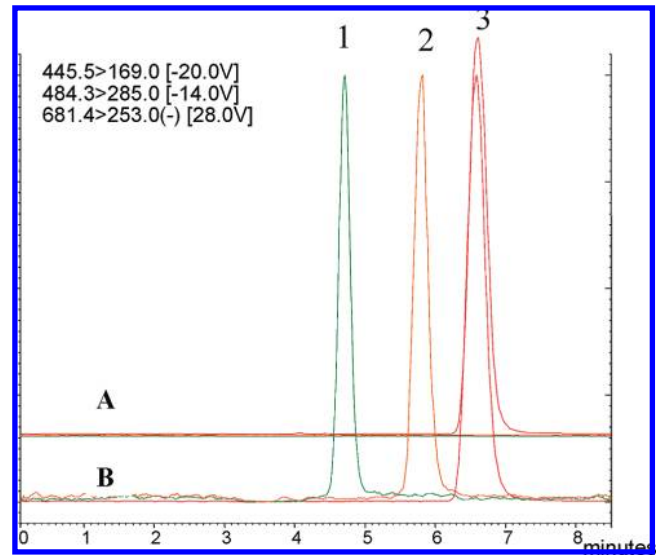

Figure 3. Ion chromatograms of (peak 1) chlorantraniliprole, (peak 2) flubendiamide, and (peak 3) internal standardL (**A**) blank of tomato; (**B**) matrix fortified at 1.6 μg/kg.

1−1000 μg/L, respectively, with $r > 0.992$; relative standard errors in slope and intercept for the diamides were below 10%. In the efficiency experiment standard solutions of each insecticide with concentrations ranging from 10 to 1000 μg/L were injected for analysis by HPLC-ESI-MS/MS in a MRM experiment. For the precision experiments the highest and lowest variation coefficients were 7.5 and 2.9%, respectively, under the conditions of repeatability, and 1.1 and 0.6%, respectively, for intraday comparisons.

For the establishment of LOQ and LOD, 1000 ng/mL standard solutions of the two diamides were gradually diluted with the mobile phase. Each individual standard was injected three times. LOD, calculated as a signal/noise ratio = 3, for CAP was 0.8 μg/kg and for FLU was 0.4 μg/kg with all matrices. LOQ, calculated as signal/noise ratio = 10, was 1.6 μg/kg for CAP and 0.8 μg/kg for FLU (**Table 2**).

Analysis of Fruits and Vegetables. Although the fruit and vegetable extracts were complex mixtures, no interfering components were detected during the chromatographic separation (**Figure 3**). The matrix effect was calculated as slope ratio

Table 3. Recoveries (Percent ± RSD; $n = 4$) of Chlorantraniliprole and Flubendiamide on Eggplant, Tomato, Pepper, Apple, Pear, And Grapes

	fortification level (μg/kg)	CAP	FLU
eggplant	1.6	103 ± 8	105 ± 8
	8	91 ± 8	87 ± 3
	16	101 ± 4	105 ± 8
	80	97 ± 6	95 ± 3
	160	93 ± 3	89 ± 1
tomato	1.6	99 ± 5	97 ± 8
	8	82 ± 5	97 ± 7
	16	83 ± 4	93 ± 7
	80	96 ± 1	101 ± 8
	160	103 ± 5	90 ± 2
pepper	1.6	92 ± 5	107 ± 9
	8	100 ± 10	91 ± 5
	16	103 ± 7	88 ± 5
	80	93 ± 3	98 ± 4
	160	90 ± 4	92 ± 2
apple	1.6	102 ± 9	104 ± 10
	8	98 ± 7	105 ± 9
	16	89 ± 2	98 ± 3
	80	96 ± 3	92 ± 4
	160	98 ± 5	96 ± 2
pear	1.6	117 ± 11	109 ± 9
	8	99 ± 3	95 ± 8
	16	109 ± 11	96 ± 7
	80	94 ± 3	93 ± 3
	160	94 ± 4	101 ± 3
grape	1.6	91 ± 8	112 ± 13
	8	100 ± 3	90 ± 7
	16	108 ± 6	112 ± 14
	80	99 ± 3	95 ± 4
	160	92 ± 4	93 ± 3

of the calibration curve prepared with the corresponding extract with the slope of the calibration curve prepared in solvent. For CAP we observed a reduction of the analytical response for all matrices. On the other hand, for FLU we found a positive matrix effect for tomatoes, eggplant, pear, and apples, with a signal enhancement of the analytical response (**Table 2**). Recoveries ranged from 82 to 117%, with coefficients of variation between 1 and 14% (**Table 3**). After sun exposure as thin layers, we observed that CAP and FLU half-life times calculated as pseudo-first-order kinetics were greater than 8 and 18 days, respectively (data not shown), indicating the possibility of persisting residues in food. For this reason we report a LC-MS/MS method in the ESI positive and negative (ion-switching) mode for the simultaneous identification and quantitation of residues of CAP and FLU in fruits and vegetables. The proposed LC-MS/MS analytical method for the determination of diamides in different food matrices has been demonstrated to be sensitive, fast, precise, accurate, and robust and can be used to monitor CAP and FLU residues in fruits and vegetables.

LITERATURE CITED

(1) Nauen, R. Insecticide mode of action: return of the ryanodine receptor. *Pest Manag. Sci.* **2006**, *62*, 690–692.

(2) Tohnishi, M.; Nakao, H.; Furuya, T.; Seo, A.; Kodama, H.; Tsubata, K. Flubendiamide, a new insecticide characterized by its novel chemistry and biology. *J. Pestic. Sci.* **2005**, *30*, 354–360.

(3) Lahm, G. P.; Selby, T. P.; Freudenberger, J. H.; Stevenson, T. M.; Myers, B. J.; Seburyamo, G. Insecticidal anthranilic diamides: a new class of potent ryanodine receptor activators. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4898–4906.

(4) Cordova, D.; Benner, E. A.; Sacher, M. D.; Rauh, J. J.; Sopa, J. S.; Lahm, G. P. Anthranilic diamides: a new class of insecticides with a novel mode of action, ryanodine receptor activation. *Pestic. Biochem. Physiol.* **2006**, *84*, 196–214.

(5) Masaki, T.; Yasokawa, T.; Tetsujoshi, N.; Tsubata, K.; Inoue, K.; Motoba, K.; Hirooka, T. Flubendiamide, a novel Ca^{2+} channel modulator, reveals evidence for functional cooperation between Ca^{2+} pumps and Ca^{2+} release. *Mol. Pharmacol.* **2006**, *69*, 1733–1739.

(6) Ebbinghaus-Kintscher, U.; Luemmen, P.; Lobitz, N.; Schulte, T.; Funke, C.; Fischer, R.; Masaki, T.; Yasokawa, N.; Tohnishi, M. Phthalic acid diamides activate ryanodine-sensitive Ca^{2+} release channels in insects. *Cell Calcium* **2006**, *39*, 21–33.

(7) http://ir4.rutgers.edu/FoodUse/newproductNOV07.pdf, last accessed May 2008.

(8) Battu, R. S.; Singh, B.; Kooner, R.; Singh, B. Simple and efficient method for the estimation of residues of flubendiamide and its metabolite desiodo flubendiamide. *J. Agric. Food Chem.* **2008**, *56*, 2299–2302.

(9) Billian, P. Residue analytical method for the determination of residues of flubendiamide and its metabolites in plant and animal materials by HPLC with electrospray MS/MS-detection. *Pflanzenschutz–Nachr. Bayer* **2007**, *60*, 263–296.

(10) Song, M.; Hang, T.; Zhao, H.; Wang, L.; Ge, P.; Ma, P. Simultaneous determination of amiloride and hydrochlorothiazide in human plasma by liquid chromatography/tandem mass spectrometry with positive/negative ion-switching electrospray ionisation. *Rapid Commun. Mass Spectrom.* **2007**, *21*, 3427–3434.

(11) Miao, X.-S.; Metcalfe, C. D. Determination of pharmaceuticals in aqueous samples using positive and negative voltage switching microbore liquid chromatography/electrospray ionization tandem mass spectrometry. *J. Mass Spectrom.* **2003**, *38*, 27–34.

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