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Trace analysis of benzalkonium chloride on skin by flow injection ionspray mass spectrometry–mass spectrometry

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A method for the analysis of trace-level benzalkonium chloride has been established using flow injection ionspray mass spectrometry–mass spectrometry with multiple reaction monitoring. Quantification was carried out using an external standard based on peak area summation of each benzalkonium ion (C_8, C_{10}, C_{12}, C_{14}, C_{16} and C_{18}) in the mass spectra. The multiple reaction monitoring technique provides additional specificity for identification and quantification. The quantification linear dynamic range was found to be 5.0–100.0 ng ml^{-1}, the correlation coefficient >0.999, and the detection limit 1.2 ng ml^{-1}. The method was applied to quantify benzalkonium chloride on skin, which was sampled with a D-SQUAME tape from skin surface and extracted from the tape with methanol.

Keywords: *Benzalkonium chloride; trace analysis; ionspray mass spectrometry; multiple reaction monitoring*

Benzalkonium (BAK) chloride is commonly used in a wide variety of health care and cosmetic preparations as an anti-microbial agent. Chemically, BAK is a mixture of alkylbenzyl-dimethylammonium ions consisting of three major homologues with straight carbon chain lengths of C_{12}, C_{14} and C_{16}. Even though BAK chloride can be safely used as an anti-microbial agent at concentrations up to 0.1%,[1] low level BAK chloride, as a result of its potential skin and ocular irritation, has been analyzed by a variety of methods such as gas chromatography,[2–4] high-performance liquid chromatography,[5–14] thin-layer chromatography,[15,16] spectrophotometry,[17–19] chemical ionization mass spectrometry,[20] fast atom-bombardment MS[21] and thermal spray LC–MS.[22] The detection limits of these methods are typically around 1–10 μg ml^{-1} or higher. The only method with a detection limit of 5 ng ml^{-1} involves an extensive sample preparation and derivatization.[8]

Soft ionization mass spectrometry, together with multiple reaction monitoring (MRM) mode detection, has found important use in the trace-level quantification for environmental samples.[23] This technique provides better specificity than other common techniques.

In the present study, it was our goal to develop a selective, sensitive and relatively simple mass spectrometric method to detect trace levels of BAK chloride on skin exposed to consumer products containing BAK chloride. Since the amount of BAK chloride transported from a product onto skin was expected to be very low, a method with a very low detection limit was needed. In this paper, we report that MRM detection provides a very low detection limit for BAK chloride and that taping with D-SQUAME tape provides a unique way to sample BAK chloride on skin.

Experimental

Reagents and supplies

All chemicals used were the purest available. Benzalkonium chloride was obtained as a 50% solution in water from Tokyo Kasei Organic Chemical Ltd. (Tokyo, Japan). HPLC-grade methanol (99.7%) and distilled water were obtained from Wako Pure Chemical Industrial Ltd. (Osaka, Japan). The calibration reagent for ionspray MS, poly(propylene glycol) (PPG), was obtained from the SCIEX API-III supplier (Concord, Ontario, Canada). It contained 3.3×10^{-5} mol l^{-1} of PPG 425, 1×10^{-4} mol l^{-1} of PPG 1000, 2×10^{-4} mol l^{-1} of PPG 2000, 2×10^{-3} mol l^{-1} of ammonium acetate, 0.1% acetonitrile and 0.1% formic acid, dissolved in a 50 : 50 solution of methanol–water.

A stock standard solution of BAK chloride (1.0 μg ml^{-1}) was prepared by dissolving the stock material in methanol. The solution was stable for at least 2 weeks if stored in the dark at 4 °C. Working standard solutions were obtained by appropriate dilution.

Sampling procedure

An appropriate amount of BAK chloride was spiked, as evenly as possible, onto one of the authors' forearms covering an area of approximately 3.8 cm^2, which was the size of the D-SQUAME tape (r = 11 mm) obtained from the Amique Group Co. Ltd. (Tokyo, Japan). After 30 min, the solvent had completely evaporated and each area was sampled with the tape (one time taping, about 5 s, pressed firmly by a thumb). The tape strips were extracted with 2 ml of methanol under vigorous shaking for 5 min. The methanol solutions were filtered through a 0.5 μm filter (Nihon Millipore Ltd., Yonezawa, Japan), and subjected to ionspray MS–MS analysis.

Flow injection ionspray mass spectrometry

The flow injection was done with a HPLC instrument (Waters 625 LC System, Nihon Millipore Ltd., Waters, Osaka, Japan) with an autosampler incorporated (Waters 717) (see Table 1). A 10 μl aliquot of extract was injected and a flow rate of 0.1 ml min^{-1} was used. The HPLC was interfaced with a PE-SCIEX API-III LC–MS–MS system (SCIEX Inc.), which is a triple quadrupole instrument, through a fused silica capillary (0.10 mm id × 0.15 mm od.

In the MRM mode, the first mass spectrometer, Q_1, was used as a mass selector by means of which all possible molecular cations from BAK mixture were isolated. Then, the isolated BAK ions were subjected to collision-induced dissociation (CID) with a collision energy of 30 eV (in the laboratory frame of reference) in Q_2. The collision gas was argon. Finally, a characteristic fragment ion from each BAK ion was selected by the third mass spectrometer, Q_3, and detected by the detector.

The mass spectrometer, both Q_1 and Q_3, was tuned and calibrated with the PPG calibration reagent with a mass accuracy of $\Delta m \leqslant 0.1$ amu over the mass range of m/z 50–2200.

Results and discussion

Study on standard solution of BAK chloride

To establish the detection limit of the method, a standard solution of BAK chloride was used. The mass spectrum of the solution of BAK chloride is shown in Fig. 1(*a*), which shows that the main components of BAK ions are homologues with C_{12}, C_{14} and C_{16} carbon chain length. The fragmentation pattern of C_{12} BAK ion in the CID is shown in Fig. 1(*b*), in which three product ions were observed. The first product ion from C_{12} BAK ion is *m/z* 212, which is formed *via* loss of toluene from the precursor ion. The fragment ion *m/z* 91 is consistent with the benzyl cation, which is produced by loss of dodecyldimethylamine from the precursor ion *via* an inductive cleavage (*i* process). The fragment ion *m/z* 58 is consistent with an immonium ion having a structure of $CH_2{=}N(CH_3)_2^+$.

The fragmentation *via* loss of toluene from the precursor ions is characteristic of all BAK ions, and therefore the product ions formed by this process were chosen as the detection ions in the MRM quantification. One of the MRM chromatograms for C_{12} BAK ion at 10.0 ng ml^{-1} is shown in Fig. 1(*c*). This MRM technique, due to high specificity, provided a lower detection limit for the analysis of BAK chloride (see below). With a direct detection of BAK ions by the first mass spectrometer (Q_1) in the selected-ion monitoring (SIM) mode, a detection limit of about 1 μg ml^{-1} was observed for the BAK chloride sampled with the tape because of the interference from the tape.

Table 1 Flow injection ionspray MS–MS conditions

Flow injection—	
Mobile phase	Methanol–water (80 + 20, v/v)
Total flow rate	0.1 ml min^{-1}
Injected volume	10 μl
Mass spectrometry—	
Ionization mode	Ionspray; positive ion
Ionspray voltage	+4800 V
Orifice voltage	+70 V
Collision energy offset	30 V
Electron multiplier voltage	−4200 V
Collision gas thickness	300×10^{12} molecules cm^{-2} (Ar)
Curtain gas flow rate	0.6 l min^{-1} (N_2)
Nebulizer gas flow rate	2.8 kg cm^{-2} (Air)
Interface temperature	60 °C
Scan mode	MRM mode
Selected ion	*m/z* 248→156, 276→184, 304→212, 332→240, 360→268, 388→296
Duration time	3.5 min
Scan rate	0.5 amu s^{-1}
Dwell time	260.0 ms
Pause time	25.0 ms

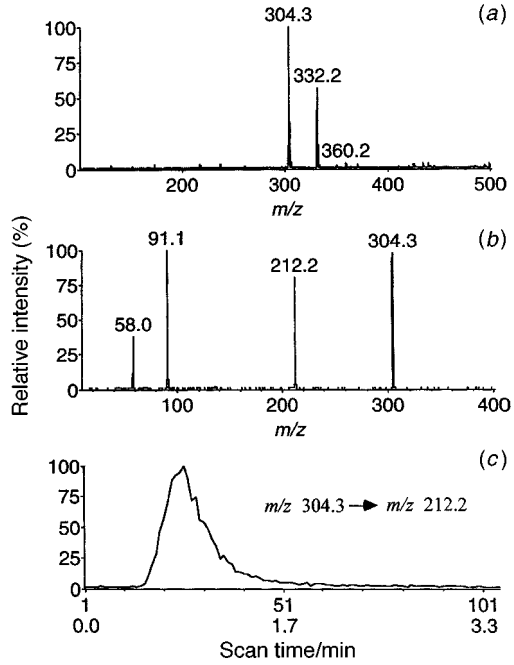

Fig. 1 (*a*), Ionspray mass spectrum of BAK chloride standard solution; (*b*), MS–MS product ion study of C_{12} BAK ion; and (*c*), MRM chromatogram of C_{12} BAK ion at 10.0 ng ml^{-1}.

Analytical parameters

The calibration curve for the standard BAK chloride samples prepared according to the procedure described above, monitored using the MRM mode, is linear for the concentration range 5.0–100.0 ng ml^{-1}. To check the linearity of the calibration curve, a linear regression equation[24] was applied to three replicates of each standard solution. Table 2 shows the results for the intercept (*a*), slope (*b*), correlation coefficient (r^2), detection limit and quantification limit. The detection and quantification limits were calculated from the calibration data according to the method proposed by Miller and Miller,[24] which takes three and ten times the standard deviation of the signal assigned to the 'zero concentration' as the signal threshold to indicate the presence and determination of an analyte for detection and quantification, respectively.

Effect of tape and skin on BAK chloride quantification

The effect of tape and skin on the recovery of the BAK chloride quantification was tested and the results are summarized in Table 3. The data show that from the tape, an average recovery of 30.6% was obtained, and from the skin, an average recovery of 17.8% was obtained. As the data reveal, the main contribution to the loss of the recovery comes from the tape.

BAK is a cation, which has a strong affinity to the anionic-natured tape and skin. This fact made it difficult to extract all the BAK chloride off the tape. However, very reproducible recoveries for both the tape and the skin enabled us to confidently quantify the BAK chloride. We observed that the response signals also have a very good linearity with the found concentrations of the BAK chloride from both the tape and the skin, which have correlation coefficients of 0.9996 and 0.9998, respectively.

Conclusions

A sensitive and practical flow injection MS–MS method for the determination of BAK chloride on skin has been established based on good linearity, precision and no interference. It is particularly applicable to the analysis of BAK chloride on the skin, with which a direct solvent extraction is difficult. The

Table 2 Analytical parameters for standard solution of BAK chloride

Intercept (*a*)	5150 counts s^{-1}
Slope (*b*)	5294 counts ml s^{-1} ng^{-1}
Correlation coefficient (r^2)	0.9999
Linear dynamic range	5.0–100.0 ng ml^{-1}
Detection limit	1.2 ng ml^{-1}
Quantification limit	3.9 ng ml^{-1}
Precision (% RSD)	≤4.1% (n = 3)

Table 3 Recovery of BAK chloride spiked on the tape and the skin

	Spiked on tape			Spiked on skin		
Concentration/ ng ml^{-1}	Found conc./ ng ml^{-1}	RSD (%)*	Recovery (%)	Found conc./ ng ml^{-1}	RSD (%)*	Recovery (%)
40.0	12.6	2.7	31.5	< 10	—	—
60.0	20.6	3.1	33.5	10.7	4.1	17.8
100.0	28.7	0.6	28.7	18.5	2.0	18.5
200.0	57.2	2.3	28.6	34.6	2.8	17.3
400.0	> 100	—	—	69.5	0.3	17.4

* The CV data were obtained with $n = 3$.

reproducible recoveries for both the tape and the skin enabled us to confidently quantify trace-level BAK chloride on skin.

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