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Surface-activated chemical ionization and high-flow gradient chromatography to reduce matrix effect

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The new atmospheric pressure chemical ionization source, named surface-activated chemical ionization (SACI), has been used in conjunction with high-flow gradient chromatography to reduce the matrix effect. This high-flow gradient chromatography approach avoids the co-elution of analyte and biological matrix compounds that leads to a reduction in quantitation errors due to matrix effect. However, this approach cannot be employed with the classical electrospray ionization (ESI) source that usually works at low eluent flow (<300 μL/min). SACI can work at high eluent flow (100–2000 μL/min) and can be employed in conjunction with high-flow gradient chromatography. The reduction in matrix effect in tacrolimus analysis in protein-precipitated blood samples, an important immunosuppressive agent for renal transplantation, is presented and discussed. Copyright © 2006 John Wiley & Sons, Ltd.

A new ionization approach, named surface-activated chemical ionization (SACI), has recently been developed.[1] This ionization source is an update of the no-discharge atmospheric pressure chemical ionization (APCI) source that has clearly shown to be in some cases more sensitive with respect to electrospray ionization (ESI) and APCI in the analysis of various compounds.[2,3] In particular, this technology has been used to analyze low to medium molecular weight compounds (peptides,[1] street drugs,[4,5] steroids,[6] and amino acids[7]). The main advantages of SACI is its capability to increase sensitivity mainly thanks to the decrease in chemical noise.[1,4–8] The physical-chemical mechanism that allows this result to be achieved has been discussed in a recent publication by Traldi and co-workers.[8] Basically, the sample solution is initially vaporized at high temperatures (300–400°C) under a nitrogen flow. At high temperature conditions the pK_a of the protic solvent (usually H_2O, CH_3OH, etc.) increases leading to the formation of $[M+nH]^{n+}$ charged species. Thus, it is possible to obtain analyte ions without using the high electrostatic potential usually employed in the case of ESI[9,10] and APCI[11,12] that leads to the production of a high amount of solvent-charged clusters that intensify the chemical noise in spectra. To increase the ionization efficiency a metallic surface placed at low electrostatic potential (50–300 V) has been inserted in the ionization chamber. It has been widely shown that this approach leads to an increase in instrumental sensitivity of about one order of magnitude, depending on the analyzed molecule and when compared to the ESI and APCI sources, respectively.[1,2,4–8]

An interesting advantage of SACI in comparison to the classical ESI is that it can work at high eluent flow rates (100–2000 μL/min). The ionization mechanism of SACI is based on the interaction of the eluent neutral species having a bipolar moment (e.g. H_2O or neutral salts CH_3COONa) with the surface so as to create a reactive environment that makes it possible to obtain the analyte ions. Moreover, the low potential applied on the surface permits better focalization of the analyte ions inside the mass spectrometer. In particular working at high flow rate the cloud of neutral reactive species surrounding the surface strongly increases leading to high ionization efficiency. This fact makes it possible to use it in conjunction with a high-flow liquid chromatography (LC) approach that leads to the reduction of the matrix effect when biological samples are analyzed.[13–16] It must be stressed that dedicated ionization sources like TurboIonspray or heated electrospray ionization (HESI) are, usually, necessary in order to work using high flow rates. APCI can also work at high LC eluent flow but it is limited to the analysis of low molecular weight compounds (usually <600 Da) while SACI

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can ionize various molecules with a wide molecular mass/charge (m/z) range (100–4000 Da).

In this work we show and discuss the benefit achieved using SACI in conjunction with high-flow gradient chromatography in terms of matrix effect reduction in the analysis of tacrolimus, an important and widely studied immunosuppressive agent, for use in renal transplantation.[17] This compound has been chosen because various approaches have been developed to quantify this molecule in plasma by using both immunoassay[17–20,23] and LC-ESI tandem mass spectrometry (MS/MS).[17–27] However, as far as we know, there is no clear discussion regarding the matrix effect problems encountered in the analysis of this compound extracted from blood by protein precipitation.

EXPERIMENTAL

Chemicals

Standard tacrolimus and ascomycin plasma samples were purchased from Chromsystem (Munich, Germany). Methanol (CH_3OH) was purchased from J.T. Baker (Deventer, The Netherlands). Formic acid (HCOOH) and sodium acetate (CH_3COONa) were purchased from Sigma Aldrich (Milan, Italy).

Sample preparation

To quantitate tacrolimus, a calibration curve was built using the following procedure. Eight standard blood samples (95 μL), with tacrolimus concentrations of 0.01, 0.1, 2.1, 5, 10.8, 15.9, 21.9 and 38.8 ng/mL, were treated with 5 μL of 100 ng/mL ascomycin standard aqueous solution to bring the concentration up to 5 ng/mL. The resulting standard blood samples were diluted (1:10) adding 900 μL CH_3OH in order to precipitate proteins. These blood samples were centrifuged and analyzed thus to achieve the calibration curves. Volume of 20 μL for analysis were injected.

To evaluate the matrix effect,[28] 15 different blood samples (100 μL for each sample) were treated with 900 μL of CH_3OH so to achieve a 1:10 blood/CH_3OH ratio. The samples were centrifuged and 90 μL of supernatant was recovered and spiked with 10 μL of a 50 ng/mL tacrolimus and ascomycin standard aqueous solution. The final concentration of tacrolimus and ascomycin was 5 ng/mL. Volumes of 20 μL per sample were injected.

The blood samples were obtained from Niguarda Hospital.

Chromatography

A Surveyor LC system (ThermoElectron, San Jose, CA, USA) was used. Two chromatographic columns were employed. The chromatographic columns were a reversed-phase Hypersil C_{18} 100 × 2.1 mm, 5 μm, 300 Å and a Hypersil C_{18} 125 × 4.6 mm, 5 μm, 300 Å. The former column was employed under isocratic conditions. The mobile phase was H_2O/CH_3OH (10:90) + 0.1% HCOOH + 0.5 mmol/L CH_3COONa. The eluent flow rate was 200 μL/min. Volumes of 20 μL were injected for each sample. The 4.6 mm i.d. column was used under high-flow gradient conditions. LC gradient was performed using two eluents: (A) H_2O + 0.1% HCOOH + 0.5 mmol/L CH_3COONa and (B) CH_3OH + 0.1% HCOOH + 0.5 mmol/L CH_3COONa. 50% of B was maintained for 3 min to eliminate the polar compounds then a fast gradient was used passing from 50% of B to 75% of B in 0.1 min. At this eluent composition the analyte eluted. 75% of B was maintained for 3 min and in 0.1 min the 90% B was reached so as to eliminate non-polar compounds. This approach avoids the co-elution of analytes and matrix compounds. These conditions were maintained for 2 min and after 0.1 min the initial conditions were reached. Thus, a run time of 8.3 min of chromatographic analysis was used but the mass chromatogram acquisition time was set to 15 min in order allow the chromatographic column to re-equilibrate. The eluent flow was 1300 μL/min. Volumes of 20 μL per sample were injected.

Mass spectrometry

The APCI and no-discharge APCI mass spectra were obtained using a LTQ (ThermoElectron, San Jose, CA, USA). The vaporizer temperature was 350°C and the entrance capillary temperature was 150°C. The corona discharge current was in the range 0–5 (0 = no-discharge APCI conditions; 5 = normal APCI) μA. A needle voltage ranging from 0 to 4 kV was monitored. The flow rate of nebulizing sheath gas (nitrogen) was 2.00 L/min. The He pressure inside the trap was kept constant; the pressure directly read by an ion gauge (in the absence of N_2 stream) was 0.7×10^{-5} Torr. The maximum injection scan time was 50 ms and 1 ms working in LC/MS/MS and direct infusion conditions, respectively. One microscan was used and the automatic gain control (AGC) was turned on during the LC/MS/MS analysis and off when operating in direct infusion conditions.

ESI spectra were obtained using a needle potential of 5 kV. The entrance capillary temperature was 275°C. The flow of nebulizing gas (nitrogen) was 1.5 L/min. The He pressure inside the trap (in the absence of N_2 stream), maximum injection scan and microscans were the same used in order to obtain the APCI spectra. The AGC was turned on during the LC/MS/MS analysis while it was turned off when acquiring direct infusion spectra.

HESI spectra were obtained using a needle potential of 2 kV. The vaporizer temperature was 200°C and the entrance capillary temperature was 275°C. The flow of nebulizing gas (nitrogen) was 2.5 L/min. The He pressure inside the trap (in the absence of N_2 stream), maximum injection scan and microscans were the same used in order to obtain the APCI spectra. The AGC was turned on during the LC/MS/MS analysis while it was turned off when acquiring direct infusion spectra.

SACI spectra were obtained using a gold surface placed at a potential of 450 V. The vaporizer temperature was 400°C and the entrance capillary temperature was 275°C. The flow of nebulizing gas (nitrogen) was 2.5 L/min. The He pressure inside the trap (in the absence of N_2 stream), maximum injection scan and microscans were the same used in order to obtain the APCI spectra. The AGC was turned on during the LC/MS/MS analysis while it was turned off when acquiring direct infusion spectra.

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LC/SACI mass chromatograms were obtained using the single reaction monitoring (SRM) tandem mass spectrometry (MS/MS) approach. The isolation width of the precursor ion was 2 mass units. The collision energy was 30% of its maximum value. One microscan was used.

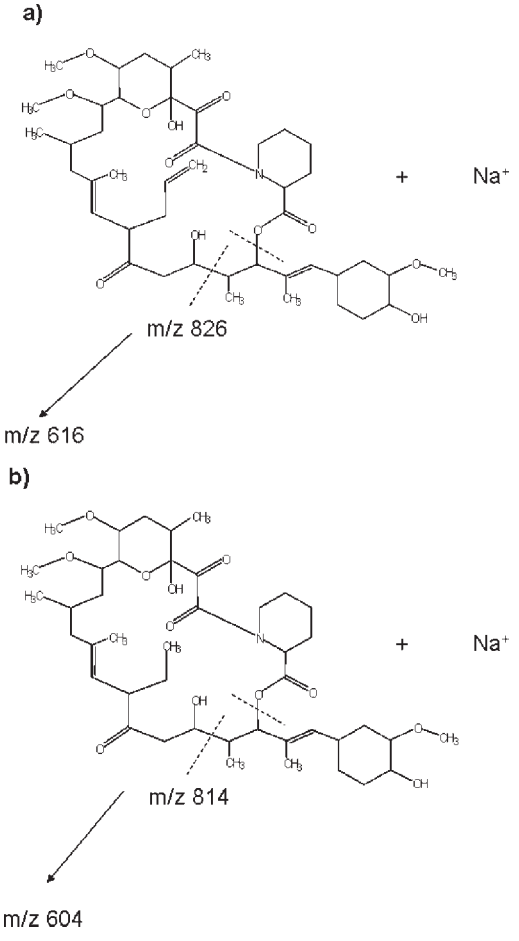
The mass spectra were acquired using positive ion mode using a sample flow range between 30 and 2000 μL/min.

Data analysis

The signal/noise (S/N) ratio was calculated using the RMS algorithm. The chromatographic data were elaborated using Xcalibur quanbrowser.

RESULTS AND DISCUSSION

Preliminary results were achieved by direct infusion of a 5 ng/mL tacrolimus (Scheme 1(a)) and ascomycin (Scheme 1(b)) solution containing 0.05 mmol/L of sodium acetate (CH_3COONa) using APCI, no-discharge APCI, ESI, HESI and SACI (Figs. 1(a)–1(e)). The direct infusion flow was 30 μL/min. As can be seen while using APCI (Fig. 1(a)), no signal was detected; in the case of no-discharge APCI (Fig. 1(b)), the $[M+Na]^+$ ions of both tacrolimus and ascomycin were clearly detected at *m/z* 826 and 814. No protonated $[M+H]^+$ ions were detected. These data are in agreement with these found in the literature.[21] These results suggest that previously observed corona discharge can lead in some cases to sample degradation especially for sodiated compounds and for compounds having medium to high molecular mass (>600 Da).[1] In the case of ESI and HESI both sodiated precursor ions of tacrolimus and ascomicine were observed (Figs. 1(c) and 1(d)) with higher counts/s values with respect to that achieved under no-discharge APCI conditions (Fig. 1(b)). However, an increase in chemical noise was also observed with respect to that obtained in the no-discharge APCI full-scan spectrum. In the case of SACI (Fig. 1(e)) an increase in sensitivity was observed in comparison to no-discharge APCI, ESI and HESI (Figs. 1(b)–1(d)). In fact the $[M+Na]^+$ ion signals of tacrolimus and ascomycin exhibit a higher counts/s value and S/N ratio (1.6×10^5 counts/s and 1010 S/N in the case of tacrolimus and 2.5×10^4 counts/s and 255 S/N in the case of ascomycin) using SACI, in comparison to those achieved using no-discharge APCI (9.1×10^2 counts/s and 20 S/N in the case of tacrolimus and 4.0×10^2 counts/s and 8 S/N in the case of ascomycin), ESI (1.0×10^4 counts/s and 30 S/N in the case of tacrolimus and 5.3×10^3 counts/s and 16 S/N in the case of ascomycin) and HESI (8.9×10^4 counts/s and 480 S/N in the case of tacrolimus and 7.1×10^4 counts/s and 290 S/N in the case of ascomycin). The same experiments were reproduced using different sample flows (100–2000 μL/min) obtaining the same results. In particular, SACI performance strongly increased by increasing the eluent flow ratio mainly due to the higher density of the reactive ionizing bipolar neutral species surrounding the surface, the good focalization of analyte ions inside the mass spectrometer and the low production of solvent charged species.[8]



Scheme 1.

The tandem mass spectra of tacrolimus and ascomycin were also achieved using SACI (Figs. 2(a) and 2(b)). The solution concentration was 5 ng/mL for each compound while the direct infusion flow was 30 μL/min. CH_3COONa was also added (0.05 mmol/L). As can be seen in the case of tacrolimus, an abundant peak at *m/z* 616 was achieved while in the case of ascomycin the most abundant ion was at *m/z* 604. The fragmentation pathways that lead to the formation of these fragments are shown respectively in Schemes 1(a) and 1(b). In Figs. 2(a) and 2(b) the neutral loss that leads to the ions at *m/z* 616 for tacrolimus and 604 for ascomycin is also reported. These ions have been used to perform a liquid chromatography single ion monitoring/surface-activated chemical ionization tandem mass spectrometry (LC-SRM/SACI-MS/MS) analysis mainly due to their high abundance and selectivity. SACI was selected for its higher sensitivity in comparison to no-discharge APCI, ESI and HESI.

Two chromatographic methods were performed and compared from the matrix effect point of view:

(i) Isocratic analysis operating at a flow rate of 200 μL/min (the eluent was H_2O/CH_3OH 10:90).
(ii) High-flow gradient chromatography (1300 μL/min).

The main advantage of the isocratic approach (i) is that it is fast and particularly suitable for a clinical laboratory that must process a high number of samples per days. In fact, under these conditions, the tacrolimus chromatographic peak elutes at a retention time of 2.81 min (Fig. 3(a)). On

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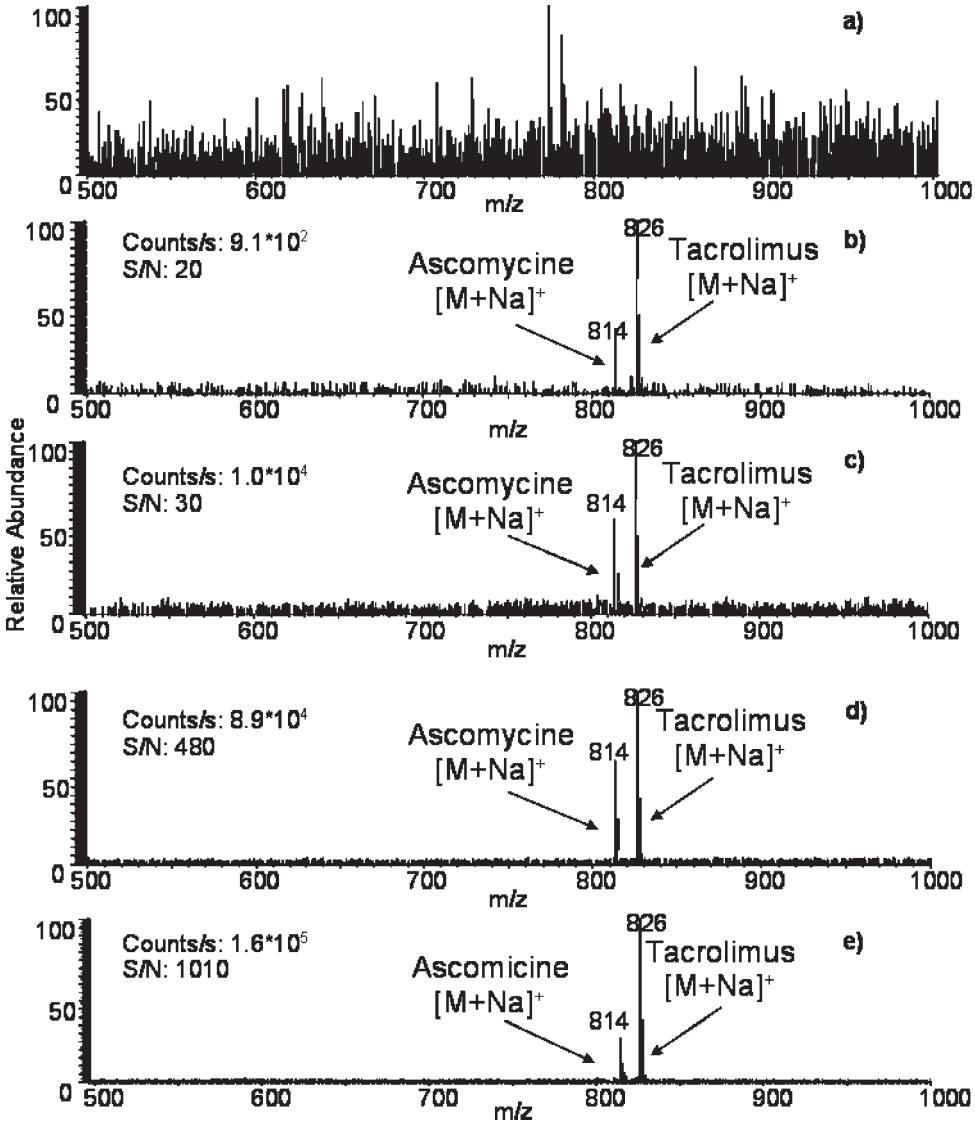

Figure 1. Direct infusion spectra obtained by analyzing a 5 ng/mL tacrolimus and ascomycin standard solution using (a) APCI, (b) no-discharge APCI, (c) ESI, (d) HESI, and (e) SACI sources. 0.05 mmol/L of sodium acetate (CH_3COONa) was added to each solution. The direct infusion sample flow was 30 μL/min. The sample solution was obtained using water as solvent. The counts/s value and the S/N ratio of the most abundant mass peak are also reported.

the other hand, the matrix molecules that are not precipitated by the blood treatment with CH_3OH can co-elute with the analyte leading to enhanced analyte signal or suppression phenomena and, consequently, to possible quantitation errors.[28] Moreover, even if an internal standard is used (ascomycin), and even if it co-elutes with the analyte (retention time 2.84 min; Fig. 3(b)), it is possible that the area of the two compounds do not co-variate, because the signal variation due to the matrix effect is different for the two compounds, leading to sample/internal standard area ratio variation and consequently to quantitation errors.[28]

In the literature a variety of achieved benefits are reported using the high-flow rapid gradient approach (ii) (ballistic chromatography, ultra-fast chromatography) to reduce the matrix effect in conjunction with ionization sources operating at high flow rates.[13–16] Thanks to the ability of SACI to operate in a wide flow range (100–2000 mL/min) and to produce ions in the same range as ESI (100–4000 Da), it becomes particularly suitable for verifying if the high eluent flow gradient approach can eliminate the matrix effect. In contrast, classical ESI cannot be employed because it usually works at a low eluent flow (<300 μL/min).

The calibration curves obtained using isocratic (i) and high-flow gradient chromatography (ii) clearly show that the linearity was definitely better using the high eluent flow rate approach ($R^2 = 0.997$) in comparison to that achieved using the isocratic low flow rate approach ($R^2 = 0.971$). This

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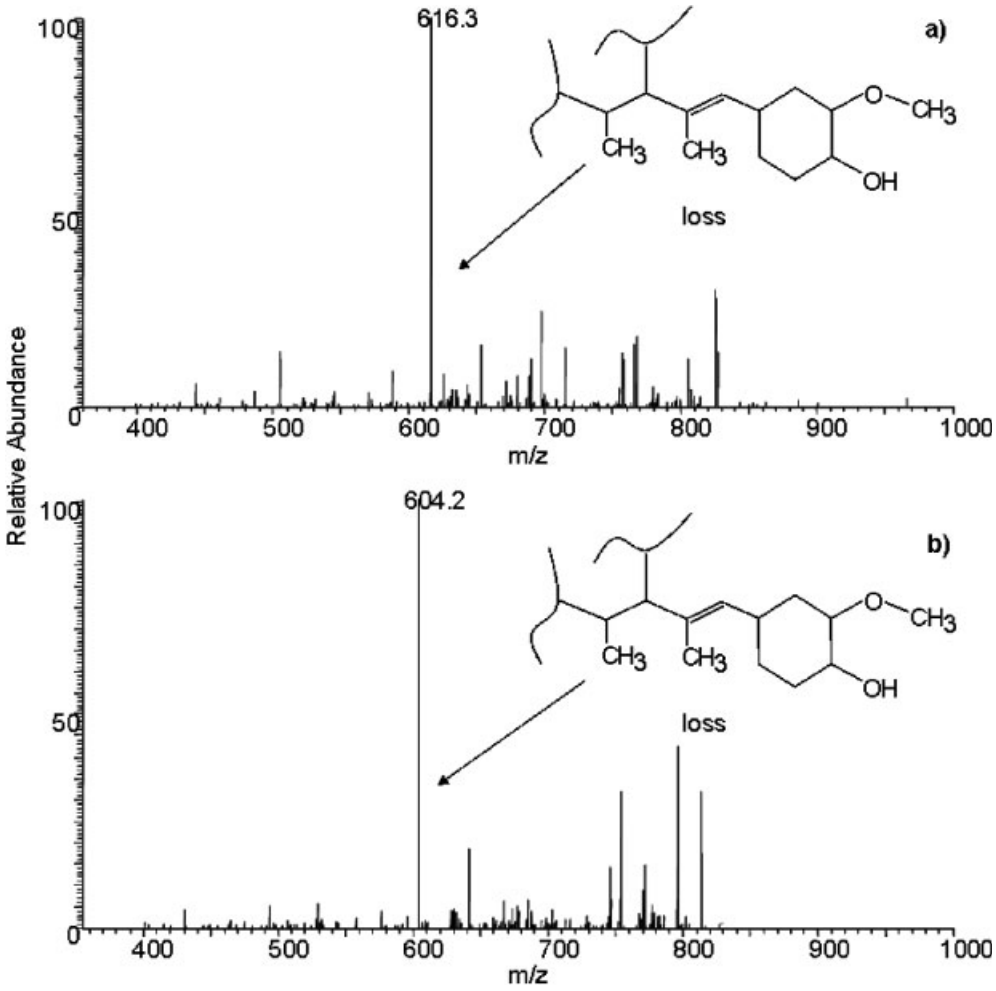

Figure 2. Direct infusion SACI tandem mass spectra of (a) tacrolimus and (b) ascomycin. The concentration was 5 ng/mL for each compound. The direct infusion sample flow was 30 μL/min. The sample solution was obtained using water as solvent. The fragments used to perform the LC-SRM/SACI-MS/MS approaches are shown together with the neutral loss that leads to their formation.

result is first evidence that the isocratic method is more affected by the matrix effect in comparison to the high-flow gradient approach. To better investigate this phenomenon 15 blood samples were subjected to protein precipitation and the supernatant was collected and tacrolimus and ascomicyn were added to it to obtain a concentration of 5 ng/mL for each compound. The two standards were added after the protein precipitation so to be sure that the analyte and internal standard chromatographic peak area variation depends only on the matrix effect and not the different sample recoveries after precipitation. LC-SRM/SACI-MS/MS mass chromatograms obtained analyzing the same sample using both the isocratic (i) and high flow gradient chromatographic (ii) approaches can be compared looking at Figs. 3a, 3b, 4a and 4b. As can be seen in both cases the chromatographic peaks of both compounds were clearly detected with high signal S/N ratio. Moreover, the S/N ratio increases by about a factor 2 using the high-flow gradient approach in comparison to the isocratic one. Table 1 reports the areas of tacrolimus and ascomycin chromatographic peaks and their ratios for the 15 protein-precipitated blood samples using the isocratic approach (i) while Table 2 reports the same results obtained using the high-flow gradient approach (ii). As can be seen in the former case both the area values and ratios are subject to higher variability (Table 1). In the case of high-flow gradient chromatography both tacrolimus and ascomycin areas and area ratios appear to be more stable (Table 2). This phenomenon is better presented in the % difference accuracy among the analyte and internal standard area ratios obtained by injecting the 15 samples and that achieved by injecting an aqueous solution (matrix effect absent) having the same analyte and internal standard concentration (5 ng/mL). The % difference accuracies are also reported in Tables 1 and 2. As can be seen while in the case of isocratic approach the % difference accuracy was in the range 3–153%, in the case of the high-flow gradient method it was in the range 2–8%. This clearly indicates that the matrix effect is strongly reduced working under high flow rate gradient conditions.

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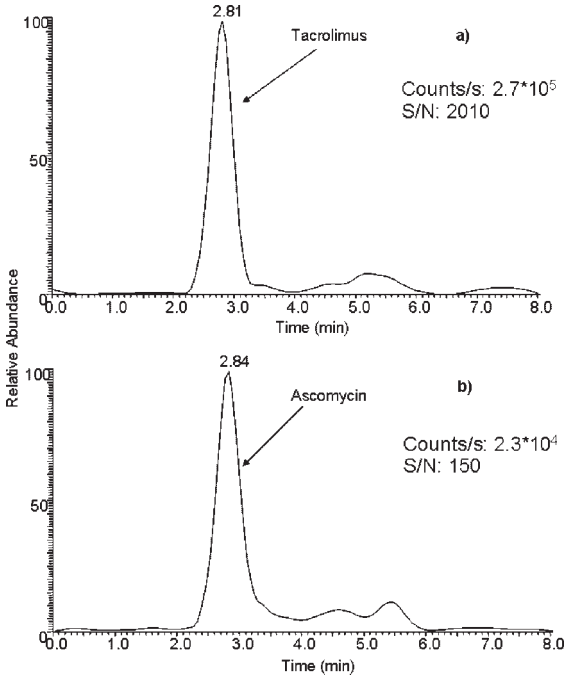

Figure 3. LC-SRM/SACI-MS/MS isocratic mass chromatograms of (a) tacrolimus and (b) ascomycin obtained by injecting 20 μL of 5 ng/mL solution of each compound in protein precipitated matrix. The eluent flow was 200 μL/min. The counts/s, peaks S/N and area are also reported.

The high flow gradient approach was so chosen to analyze 50 blood samples containing tacrolimus and ascomycin at various known concentrations. As an example the theoretical and calculated tacrolimus concentration, the % difference accuracy and the precision expressed in %CV of 15 representative blood samples are reported in Table 3. As can be seen, the quantitation accuracy, expressed as % difference accuracy, was between 2 and 8% and the precision, expressed as % CV, was lower than 5% and these ranges were obtained for all 50 analyzed compounds.

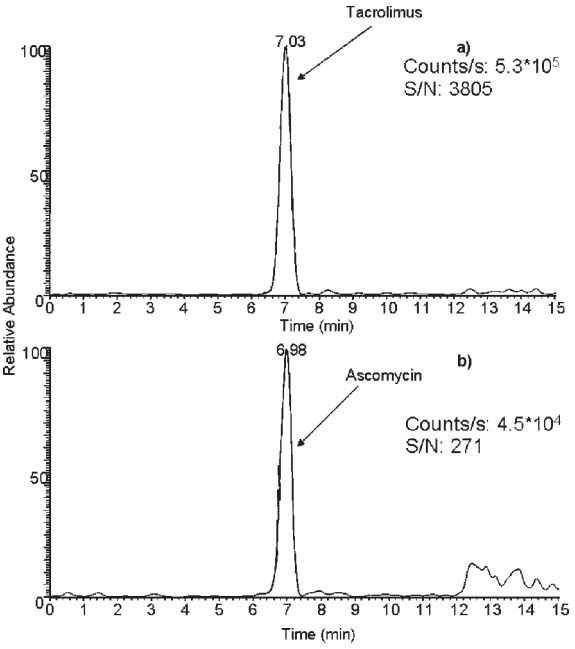

Figure 4. LC-SRM/SACI-MS/MS high flow gradient mass chromatograms of (a) tacrolimus and (b) ascomycin obtained by injecting 20 μL of 5 ng/mL solution of each compound in protein-precipitated matrix. The eluent flow was 1300 μL/min. The counts/s, peaks S/N and area are also reported.

Table 1. Tacrolimus and ascomycin chromatographic peaks areas, area ratios and % difference accuracy for the 15 protein-precipitated blood samples, obtained using the isocratic approach

Samples	Area tacrolimus peak	Area internal standard	Ratio	Ratio % difference accuracy
1	16125	14256	1.13	3
2	26352	11256	2.34	112
3	17256	10256	1.68	53
4	16256	13260	1.23	11
5	17289	14586	1.19	8
6	15286	9285	1.65	49
7	20585	11256	1.83	66
8	18386	6582	2.79	153
9	15798	12526	1.26	14
10	25859	16574	1.56	42
11	21584	15254	1.41	28
12	19856	13241	1.50	36
13	18956	17856	1.06	4
14	13859	8596	1.61	46
15	14589	12578	1.16	5

CONCLUSIONS

In conclusion a strong matrix effect reduction was observed working with high-flow gradient chromatography coupled with SACI. It must be emphasized that this result has been achieved mainly thanks to the ability of SACI to operate at

Table 2. Tacrolimus and ascomycin chromatographic peaks areas, area ratios and % difference accuracy for the 15 protein-precipitated blood samples, obtained using the high-flow gradient approach

Samples	Area tacrolimus peak	Area internal standard	Ratio	Ratio % difference accuracy
1	32250	28512	1.13	3
2	31718	28300	1.12	2
3	34512	33780	1.02	7
4	32512	28280	1.15	4
5	34202	28630	1.19	8
6	30572	28316	1.08	2
7	35978	33900	1.06	4
8	37918	35242	1.08	2
9	31780	29974	1.06	4
10	32428	30562	1.06	4
11	35702	33050	1.08	2
12	35578	30578	1.16	6
13	36312	35712	1.02	8
14	27718	25774	1.08	2
15	30516	29574	1.03	6

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Table 3. Tacrolimus calculated and theoretical quantitation data, %CV precision and % difference accuracy for the 15 protein-precipitated blood samples reported as representative data from a pool of 50 samples

Samples	Calculated tacrolimus concentration (ng/mL)	Theoretical tacrolimus concentration (ng/mL)	%CV precision	Ratio % difference accuracy
1	11.5	11.3	2	2
2	5.6	5.4	4	4
3	8.2	7.8	4	5
4	14.2	13.7	3	4
5	12.1	11.5	4	5
6	3.2	3.0	4	8
7	10.3	10.6	2	3
8	6.2	5.9	4	5
9	7.9	7.5	4	6
10	7.5	7.1	3	5
11	13.1	13.7	2	4
12	16.5	15.9	2	4
13	9.7	9.2	3	5
14	9.4	8.9	3	6
15	10.1	10.2	2	2

high flow rates. Thus, SACI can be considered as a potential alternative to the API sources operating at high solvent flow rates (APCI, TurboIonspray, and HESI).

Future development will be focused on developing new high-flow gradient methods to be used to reduce the matrix effect in analysis of other compounds of clinical interest.

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REFERENCES

1. Cristoni S, Bernardi LR, Biunno I, Tubaro M, Guidugli F. *Rapid Commun. Mass Spectrom*. 2003; **17**: 1973.
2. Turnipseed SB, Andersen WC, Karbiwnyk CM, Roybal JE, Miller KE. *Rapid Commun. Mass Spectrom*. 2006; **20**: 1231.
3. Cristoni S, Bernardi LR, Biunno I, Guidugli F. *Rapid Commun. Mass Spectrom*. 2002; **16**: 1686.
4. Cristoni S, Bernardi LR, Gerthoux P, Gonella E, Mocarelli P. *Rapid Commun. Mass Spectrom*. 2004; **18**: 1847.
5. Cristoni S, Cantu M, Bernardi LR, Gerthoux P, Mocarelli P, Brambilla M, Gonella E, Guidugli F. *J. Mass Spectrom*. 2005; **40**: 1609.
6. Cristoni S, Sciannamblo M, Bernardi LR, Biunno I, Gerthoux P, Russo G, Chiumello G, Mora S. *Rapid Commun. Mass Spectrom*. 2004; **18**: 1392.
7. Cristoni S, Cantu M, Bernardi LR, Gerthoux P, Gonella E, Brambilla M, Cavalca V, Zingaro L, Guidugli F. *Rapid Commun. Mass Spectrom*. 2005; **19**: 1231.
8. Cristoni S, Bernardi LR, Guidugli F, Tubaro M, Traldi P. *J. Mass Spectrom*. 2005; **40**: 1550.
9. Whitehouse CM, Dreyer RN, Yamashita M, Fenn JB. *Anal. Chem*. 1985; **57**: 675.
10. Fenn JB. *Angew. Chem. Int. Ed. Engl*. 2003; **42**: 3871.
11. Horning EC, Carroll DI, Dzidic I, Haegele KD, Horning MG, Stillwell RN. *J. Chromatogr. Sci*. 1974; **12**: 725.
12. Carroll DI, Dzidic I, Stillwell RN, Haegele KD, Horning EC. *Anal. Chem*. 1975; **47**: 2369.
13. Tiller PR, Romanyshyn LA. *Rapid Commun. Mass Spectrom*. 2002; **16**: 92.
14. Romanyshyn LA, Tiller PR. *J. Chromatogr. A*. 2001; **928**: 41.
15. Deng Y, Zhang H, Wu JT, Olah TV. *Rapid Commun. Mass Spectrom*. 2005; **19**: 2929.
16. Ceglarek U, Lembcke J, Fiedler GM, Werner M, Witzigmann H, Hauss JP, Thiery J. *Clin. Chim. Acta* 2004; **346**: 181.
17. Borrows R, Chusney G, Loucaidou M, Singh S, James A, Stichbury J, Van Tromp J, Cairns T, Griffith M, Hakim N, McLean A, Palmer A, Papalois V, Taube D. *Transplant. Proc*. 2005; **37**: 1733.
18. Braun F, Lorf T, Schutz E, Christians U, Grupp C, Sattler B, Canelo R, Sewing KF, Armstrong VW, Oellerich M, Ringe B. *Transplant. Proc*. 1996; **28**: 3175.
19. Salm P, Taylor PJ, Clark A, Balderson GA, Grygotis A, Norris RL, Lynch SV, Shaw LM, Pond SM. *Ther. Drug Monit*. 1997; **19**: 694.
20. Armstrong VW, Schuetz E, Zhang Q, Groothuisen S, Scholz C, Shipkova M, Aboleneen H, Oellerich M. *Clin. Chem*. 1998; **44**: 2516.
21. Lensmeyer GL, Poquette MA. *Ther. Drug Monit*. 2001; **23**: 239.
22. Streit F, Armstrong VW, Oellerich M. *Clin. Chem*. 2002; **48**: 955.
23. Gonschior AK, Christians U, Winkler M, Schiebel HM, Linck A, Sewing KF. *Ther. Drug Monit*. 1995; **17**: 504.
24. Taylor PJ, Jones A, Balderson GA, Lynch SV, Norris RL, Pond SM. *Clin. Chem*. 1996; **42**: 279.
25. Taylor PJ, Hogan NS, Lynch SV, Johnson AG, Pond SM. *Clin. Chem*. 1997; **43**: 2189.
26. Volosov A, Napoli KL, Soldin SJ. *Clin. Biochem*. 2001; **34**: 285.
27. Ramakrishna NV, Vishwottam KN, Puran S, Manoj S, Santosh M, Wishu S, Koteshwara M, Chidambara J, Gopinadh B, Sumatha B. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci*. 2004; **805**: 13.
28. Mallet CR, Lu Z, Mazzeo JR. *Rapid Commun. Mass Spectrom*. 2004; **18**: 49.

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