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On the use of liquid chromatography with radio- and ultraviolet absorbance detection coupled to mass spectrometry for improved sensitivity and selectivity in determination of specific radioactivity of radiopharmaceuticals

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Abstract

Pneumatically assisted electrospray mass spectrometry was evaluated as a complementary detection technique to UV absorbance, for determination of specific radioactivity of tracer molecules to be used in positron emission tomography. Tracers labelled with radionuclides having short half-lives can be synthesised with high specific radioactivity. The UV absorbance detection that is commonly used for the determination does not always have the sensitivity required for those analyses. In comparison, mass spectrometry gave improved detection limits in all but one (nicotine) of the 12 compounds studied. The magnitude of this improvement was more than 100-fold for the compounds ketamine (2-methylamino-2-(2-chloro-phenyl)cyclohexanone), SCH-23390 ((*R*)-(+)-7-chloro-8-hydroxy-1-methyl-1-phenyl-2,3,4,5-tetra-hydro-1*H*-3-benzazepine) and *N*-methyl-piperidylbenzilate. These improved detection limits, specificity, plus the added certainty of product identity provided by mass spectral data demonstrated the value of the mass spectrometer as a complementary detector in the determination of specific radioactivity.

Keywords: Liquid chromatography; Mass spectrometry; Specific radioacitivity; Carbon-11

1. Introduction

Biochemical and physiological processes can be studied in vivo using pharmaceuticals labelled

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with short-lived β^+-emitting radionuclides (e.g. ^{11}C, $t_{1/2} = 20.3$ min). The distribution of radioactivity in the body is monitored as a function of time by positron emission tomography (PET) [1]. Because of the short half-life of the radionuclide, synthesis and analysis of labelled tracers/pharmaceuticals must be quick and performed prior to every investigation. In the synthesis of ^{11}C-labelled compounds, it is important to obtain the lowest possible concentration of the ^{12}C analogue. A measure of the ratio between the radiolabelled and the unlabelled compound is the specific radioactivity (Bq/mol). The total quantity of tracer, which is administrated to the subject, can be determined from the specific radioactivity and the amount of radioactivity distributed. In order to perform a study with negligible effects of the system studied, it is essential that this amount is low. This data of the tracer concentration at the time of administration can thus be used for correcting PET data to improve the value of the investigation. Determination is commonly performed by measurement of the radioactivity of the isolated product combined with its quantification using liquid chromatography (LC) separation with UV absorbance detection. Product identities are confirmed by matching retention times with those of standards. When high specific radioactivity is obtained, it puts higher demands on these determinations and it is not uncommon that quantification may be performed close to the detection limit of the system. The aim within PET methodology is to reach even higher specific radioactivity, which theoretically and practically should be possible. The use of a complementary detector, which provides better sensitivity for such analyses could be of great value in the PET environment.

The atmospheric pressure ionisation (API) techniques, such as electrospray (ESI) and atmospheric pressure chemical ionisation (APCI), have significantly increased the availability of mass spectrometry as a chromatographic detector [2]. The ability to determine molecular weights and sequence information of very large molecules has been extensively investigated. Another application has been in the development of selectivity and sensitivity in the quantitative analysis of smaller molecules. This is commonly performed by combining liquid chromatographic separation with mass spectrometric detection (LC–MS) [3,4]. The use of API–MS for detection is known to afford high sensitivity in analysis [5,6] and was therefore an interesting alternative to UV absorbance detection for determination of specific radioactivity.

In this study, pneumatically assisted electrospray mass spectrometry was evaluated as a supplementary detection technique to UV absorbance for a number of commonly encountered PET tracers. The same separation system routinely used for quantification with UV absorbance detection was modified with a 1:100 split.

2. Experimental

2.1. Materials and reagents

Formic acid and trifluoroacetic acid (TFA) of analytical grade were from E. Merck (Darmstadt, Germany). Acetonitrile of ultra gradient grade from Fisons (Loughborough, UK) and nanopure water (Elga Maxima, Bucks, UK) were used as mobile phases.

7-Methoxy-1-methyl-9*H*-[3,4-b]indole (harmine) and 3-(1-methyl-pyrrolidin-2-yl)-pyridine (nicotine) were purchased from Sigma (St Louis, USA). 3,5-Dichloro-6-hydroxy-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-2-metoxibenzamide (raclopride), 3,5-dichloro-2,6-hydroxy-N-[(1-ethyl-2-pyrrolidinyl)-methyl]benzamide (desmethyl-raclopride) and (*S*)-5-bromo-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-2,3-dimetoxibenzamide (FLB 457) were obtained from Astra Arcus (Södertälje, Sweden). Ethyl-8-fluoro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-a]-[1,4]benzodiazepine-3-carboxylate (Ro 15-1788) and ethyl-8-azido-5,6-dihydro-5-methyl-6-oxo-4-*H*-imidazo[1,5-a]-[1,4]benzodiazepine-3-carboxylate (Ro 15-4513) were obtained as a gift from Dr DaPrada at Hoffman-La Roche (Basle, Switzerland). *N*-Methyl-8-[4-(4-fluorophenyl)-4-oxobutyl]-1-phenyl-1,3,8-triazaspiro-[4,5]decan-4-one (*N*-methyl-spiperone) and (*R*)-(+)-7-chloro-8-hydroxy-1-methyl-1-phenyl-2,3,4,5-tetra-hydro-1*H*-3-benzazepine (SCH-23390) were from RBI

(Massachusetts, USA). Methyl-(1-methyl-2-phenyl-ethyl)-prop-2-ynyl-amine (deprenyl) was from Orion Farmos OY (Espoo, Finland) and 2-methylamino-2-(2-chloro-phenyl)cyclohexanone (ketamine) from Parke-Davis (New Jersey, USA).

N-Methyl-piperidylbenzilate [7] and (5*S*,10*R*)-(−)-3-cyano-5-methyl-10,11-dihydro-5*H*-dibenzo[a,d]cyclohepten-5,10-imine ((+)-3-cyano-MK-801) [8] were synthesised in-house according to published procedures.

2.2. Liquid chromatography

Mobile phases were (A) 5 mM formic acid or TFA in water, and (B) 5 mM formic acid or TFA in acetonitrile. A Beckman 126 solvent delivery module (Beckman Instruments, Fullerton, CA, USA) was run at a flow rate of 1 ml/min. Samples were injected with CMA autosampler (CMA/Microdialys AB, Stockholm, Sweden) and all analyses were performed on a 100 × 4.6 mm I.D. Kromasil 5 μm C18 column (Phenomenex, Torrance, CA, USA). A column heater (Microlab, Aarhus, Denmark) was operated at 40°C. A post column split delivered 10 μl/min to the mass spectrometer and 990 μl/min to UV absorbance detection with a β^+-flow detector [9] in series.

Isocratic analyses were performed with additions of organic modifier according to Table 1. Raclopride and deprenyl showed pronounced tailing during isocratic conditions and were therefore analysed with linear gradients (Table 1).

2.3. Mass spectrometry

The mass spectrometer was a Fisons VG Platform (Micromass UK, Altrincham, Cheshire, UK) equipped with pneumatically assisted electrospray and a RF-ion bridge in the second vacuum stage. Selected ion recording (SIR) was performed on each individual mass. Determination of the different analytes was performed on the mass corresponding to $[M+H]^+$ at the m/z values shown in Table 1. Optimisation of parameters such as spray voltage, interface potentials, probe position, temperature and gas flows were performed for every analyte during direct infusion. The mass spectrometer was tuned to unit mass resolution.

2.4. UV absorbance and radiodetection

A Beckman 166 UV absorbance detector was connected in series to a β^+-flow detector [9]. In order to obtain the highest sensitivity, the optimum wavelengths for each analyte were determined (Beckman DU 7500 spectrophotometer). Eight analytes had absorbance maximum above 240 nm. These analytes were analysed at their respective optimal wavelengths (Table 1) and the

Table 1
Experimental LC–UV–MS parameters for detection of 12 commonly used PET tracers

	Solvent (% acetonitrile)	Modifier (5 mM)	Selected ion[a] (m/z)	Absorbance λ (nm)
(+)-3-Cyano-MK-801	20	TFA/formic	247.1	254
Deprenyl	15–45[b]	TFA	188.1	258
FLB 457	30	TFA	371.1	254
Harmine	20	Formic acid	213.1	248
Ketamine	20	TFA	238.1	270
Nicotine	5	TFA/formic	163.1	259
N-Methyl-piperidylbenzilate	30	Formic acid	326.1	254
N-Methyl-spiperone	35	TFA	410.2	248
Raclopride	30–70[b]	TFA/formic	347.1	254
Ro 15-1788	30	Formic acid	304.1	242
Ro 15-4513	35	Formic acid	327.1	269
SCH-23390	25	TFA	287.1	282

[a] m/z used in selected ion recording (SIR).
[b] Linear gradient during 3 min.

Table 2
Detection limits[a] in nM for a 20 μl sample injection by UV-absorbance detection

	200 nm	210 nm	220 nm	230 nm
(+)-3-Cyano-MK-801	8.5	15	15	21
Deprenyl	320	270	530	
FLB 457	28	23	33	60
Ketamine	74	130	180	670
N-Methyl-piperidylbenzilate	22	22	28	63
Raclopride	22	16	22	34
SCH-23390	11	13	28	48

[a] Peak height of three times the peak-to-peak noise.

remaining analytes at 254 nm. Seven of the compounds showed improved absorbance at wavelengths below 240 nm (Table 2). Analysis in the low wavelength region can be performed, although it puts higher demands on degassing of solvents, low amounts of impurities, etc. It may thus be difficult to perform a satisfactory purity control of the product in the low wavelength region on a routine basis.

2.5. Reproducibility and noise

To determine the precision in the detection limits, the peak height was measured at low concentrations. The relative standard deviation (RSD) was 3% for six repetitive injections and this precision was equal for all measurements. The noise level for UV absorbance detection was constant at a value of $2e-5$ absorbance units for all the reported analyses, while the noise level was changing at different m/z values for the mass spectrometry data (Table 3) due to changes in the chemical background.

3. Results and discussion

In work with short-lived radionuclides, the time available for the analysis is limited by the physical half-life of the radionuclide. The compounds in this study were all labelled with ^{11}C, with a half-life of 20.3 min. Hence, there was a need for rapid analysis with high sensitivity. A LC system with radiodetection in combination with UV absorbance and mass spectrometry, has been utilised for its speed and sensitivity in determining if the desired compound has been labelled [8,10] by correlation of the radioactive compound with a molecular mass or a spectrum [11,12].

The investigated compounds, exemplified in Fig. 1, were all moderately polar and weakly basic in character and therefore readily separated by reversed phase chromatography. The same separation column was used throughout the study and all compounds were retained with a $k' > 2$, giving a retention time of some three minutes for all analyses, except for nicotine ($k' \sim 1$).

The column eluent was directed through three different detectors. Since the best signal-to-noise ratio in pneumatically assisted electrospray ionisation is observed at low flow rates [2,13], the eluent was routed through a 1:100 split, so that 10 μl/min went to the mass spectrometer and 990 μl/min to radio- and UV absorbance detection. The selectivity and sensitivity by LC–MS was evaluated using this instrumental set-up.

Electrospray mass spectrometry requires volatile mobile phase additives for stable operation. Formic acid and TFA are commonly used for this reason and were both evaluated. TFA is often preferred in reversed-phase separations since this strong acid forms ion pairs with the basic compounds, resulting in increased retention times and improved peak shapes [14]. This was also observed for the analytes included in this study, as they all eluted at the same or longer retention time, when formic acid was exchanged for TFA. In addition, the peak shapes of raclopride [15] and deprenyl [16] were significantly improved when TFA was used as additive.

Table 3
Detection limits[a] for a 20 μl injection after a post-column[b] split of 1:100 to the MS and UV detection

	UV[c] (nM)	MS[d] (nM)	Noise MS (cps × 10^3)
(+)-3-Cyano-MK-801	60	8.1[e,f]	9
Deprenyl	2700	27[f]	3
FLB 457	220	5.4[f]	5
Harmine	94	4.7[e]	6
Ketamine	1300	11[f]	5
Nicotine	200	200[e,f]	30
N-Methyl-piperidylbenzilate	680	3.1[e]	6
N-Methyl-spiperone	61	2.4[f]	4
Raclopride	87	5.8[e,f]	4
Ro-15-1788	12	9.9[e]	4
Ro-15-4513	21	21[f]	10
SCH-23390	380	3.5[f]	20

[a] Peak height of three times the peak-to-peak noise.
[b] 4.6 × 100 mm reversed phase column.
[c] 990 μl/min from the LC column.
[d] 10 μl/min from the LC column.
[e] 5 mM formic acid as buffer.
[f] 5 mM TFA as buffer.

It has been found that the use of TFA, however, can cause significant signal suppression in electrospray ionisation as a consequence of its ability to form strong ion pairs [14,17,18]. All analytes were therefore analysed with both formic acid and TFA for comparison. As seen in Table 4, *N*-methylspiperone [19], ketamine [20] and SCH-23390 [21] showed between two and six times lower detection limits in the TFA buffer while raclopride, nicotine [22], (+)-3-cyano-MK-801 [8], deprenyl and FLB 457 [23] showed a similar sensitivity in both buffers. It can therefore be concluded that signal suppression by TFA is not a limiting factor for these analytes. For weakly basic molecules, a stronger signal can rather be observed with addition of TFA, due to more efficient protonation in solution [14]. The four substances, harmine [24], Ro 15-1788 [25], Ro 15-4513 and *N*-methyl-piperidylbenzilate [7], showed two to four times better detection limits with formic acid.

It is known that the electrospray ionisation process works well with analytes that exist as ions or ion–molecule complexes in solution. The compounds included in this study all contained amine groups and were subsequently easy to detect in positive ESI–MS (Fig. 1). The protonated molecule $[M+H]^+$ was typically the most abundant ion in the spectrum. The limited fragmentation results in high sensitivity per m/z value and as a result, selected ion recording (SIR) on the molecular ion could be performed on concentrations in the low nM range (20 μl injection).

The improved selectivity that was obtained by use of mass spectrometry in combination with radiodetection provided solutions to some general difficulties associated with synthesis of tracers for use in PET. One example is found in the analysis of the labelled product. The syntheses of most of the radiolabelled molecules were performed by methylation reactions of the corresponding desmethyl compounds. Purification of the radiolabelled compound was performed by semi-preparative reversed-phase high-performance liquid chromatography (HPLC). The substrate and the product had similar structures and were often eluting with poor resolution in this purification process. When the ratio of the remaining substrate to the product was large, the product fraction was collected on the slope of the preceding peak. The final product solution then naturally might contain some substrate. When the results from a PET study are evaluated it is helpful to know the quantity of this remaining non-labelled

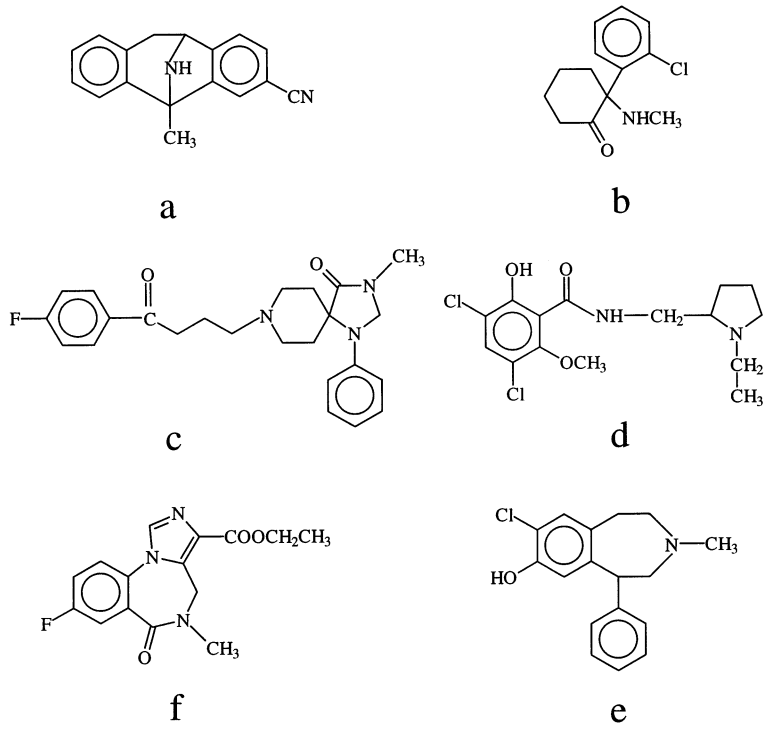

Fig. 1. Examples of structures; (a) (+)-3-cyano-MK-801, (b) ketamine, (c) *N*-methyl-spiperone, (d) raclopride, (e) Ro 15-1788 and (f) SCH-23390.

know the quantity of this remaining non-labelled precursor. By using the m/z difference between the two compounds they could be separated in the mass analyser and therefore quantification could be performed even with peak overlap.

When quantification is performed on coeluting analyte peaks ion suppression may occur in ESI-MS, which can reduce the ion intensity and thereby effect quantitative reproducibility [26,27]. The sample matrix of the synthetic product should contain few unwanted components, except for remaining substrate, since the product is purified and characterised prior to these analyses. As the sample impurities are known, the possible ion suppression that occurs can be investigated, for each analyte of interest. The synthetic product of ^{11}C-raclopride often contains low concentrations of the non-labelled precursor. Possible ion suppression between these two compounds was therefore investigated by flow-injection analyses and determination of the peak area. Raclopride and desmethyl-raclopride were first injected separately in 5 mM formic acid solutions an determined at seven different concentrations by selected ion monitoring (SIM) of the protonated molecules (m/z 347 and 333, respectively). New solutions were made by mixing the two compounds at equal concentrations. The analyte responses were then determined again for each compound. As can be seen in Fig. 2, the responses at concentrations below 7 μM were not affected by the matrix component. At higher concentrations desmetyl-raclopride showed a decreased signal response when raclopride was present in the solution. It was therefore concluded that quantification of these two compounds could be performed without significant ion suppression, at analyte concentrations below 7 μM even when peaks coelute in the chromatogram.

Another situation when selectivity can be critical is in determination of specific radioactivity. This may be performed after synthesis, by quan-

tification of the analyte peak in a UV absorbance chromatogram combined with radioactivity measurement. As the radiolabelled tracers can be synthesised with a high specific radioactivity, the concentration of the product solution becomes very low. Some analytes, such as ketamine, show very poor UV absorbance. Even minor impurities that absorb UV light at the same wavelength, seriously disturbs the quantification. By analysis with LC–radio-MS, quantification of ^{11}C-ketamine [20] could be performed in the mass chromatogram without disturbances from overlapping peaks. As can be seen in Fig. 3, the mass could also be correlated to the radioactivity chromatogram for identification of the labelled compound. It is also well known that the theoretical specific radioactivity for these compounds is much higher. In the synthetic work, the aim is to achieve the highest specific radioactivity possible and thus detection techniques which can provide high sensitivity are required in order to evaluate such work. In this study, it was, therefore, also of interest to determine how much better sensitivity electrospray mass spectrometry could provide.

The sensitivity was compared between UV absorbance and mass spectrometry detection for 12 selected compounds of interest. The detection limit was defined as a peak height of three times the peak-to-peak noise. The mass spectrometer showed significantly lower detection limits for all analytes, except nicotine, when compared to the UV absorbance detection (Table 3). The substances that showed the lowest sensitivity in UV absorbance detection, such as *N*-methyl-piperidylbenzilate, deprenyl, ketamine and SCH-23390, showed the best sensitivity improvement when analysed with mass spectrometry. *N*-methyl-piperidylbenzilate, ketamine and SCH-23390 showed the greatest difference in detection limits, with more than 100 times better sensitivity in the mass spectrometer. The detection limit for nicotine was high in the mass spectrometer, as compared with the other compounds. As can be seen in Table 3, the background in the mass spectrometer is not constant over the m/z range. At m/z 163, where nicotine was detected, the background was unusually high, possibly due to a cluster ion of water. It might be possible to improve the signal-to-noise ratio in analysis of nicotine by MS/MS analysis, as the background would be reduced.

Table 4
Detection limits[a] for a 20 μl injection in LC–MS analysis using 5 mM TFA and 5 mM formic acid, respectively, as buffer additives

	TFA (nM)	Formic acid (nM)
(+)-3-Cyano-MK-801	8.1	8.1
Deprenyl	27	32
FLB 457	5.4	8.1
Harmine	9.4	4.7
Ketamine	11	21
Nicotine	200	200
N-Methyl-piperidylbenzilate	9.2	3.1
N-Methyl-spiperone	2.4	15
Raclopride	7.2	5.8
Ro-15-1788	26	9.9
Ro-15-4513	86	21
SCH-23390	3.5	7.0

[a] Peak height of three times the peak-to-peak noise.

The specific radioactivity was determined on the synthetic products of the tracer molecules described. Quantification was performed with UV absorbance detection as well as electrospray mass spectrometry, by use of the same calibration standards. The quantitative results from the two methods were, as expected, the same. The linear dynamic range in ESI–MS typically reached up to 10^{-5} M, in agreement with what has previously been reported [28,29]. The peak height was measured to determine the precision in analysis and it was found that the relative standard deviation (RSD) was 3% for six repetitive injections when determined at concentrations close to the detection limit, and was the same for both detectors. In the samples with highest specific radioactivity, however, UV absorbance could not be used for detection. As a result, all the ketamine and (+)-3-cyano-MK-801 determinations had to be performed with electrospray mass spectrometry.

Sensitivity in analysis can thus be significantly enhanced by use of ESI–MS detection in analysis, although the majority of the analytes never enters the ionisation source. Flow splitting can be performed without loss of sensitivity, since electrospray mass spectrometry performs as a concentration sensitive detection technique [2,30]. However, if the

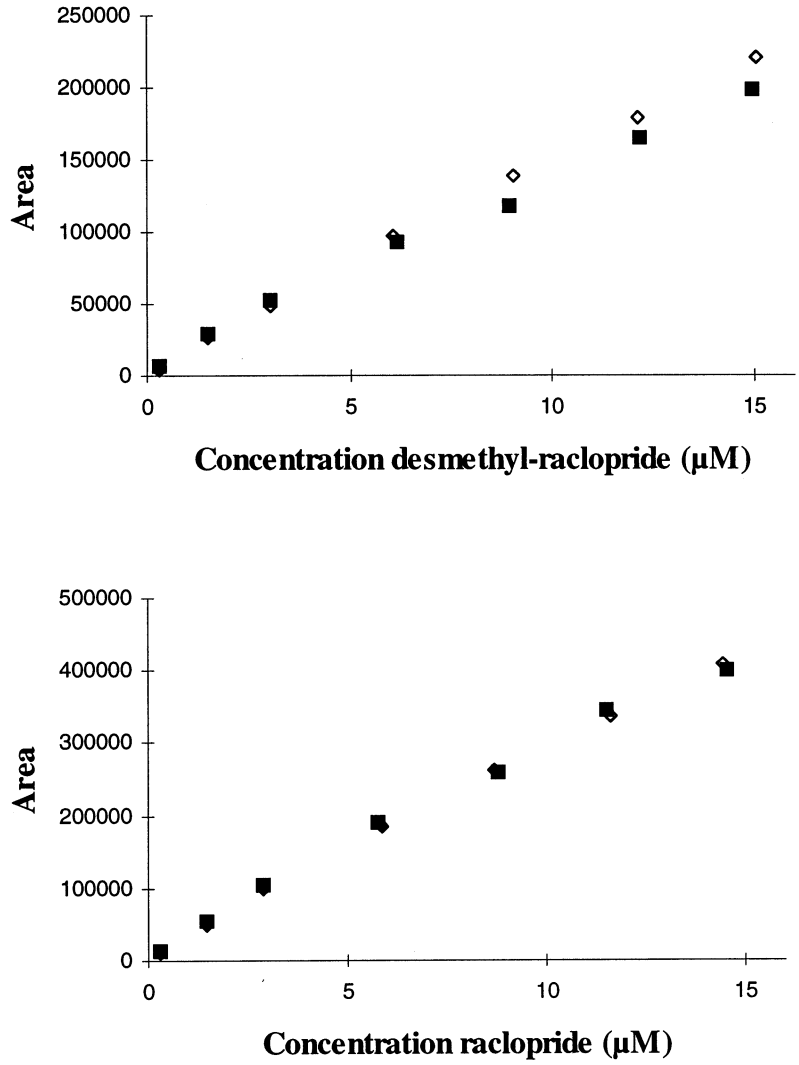

Fig. 2. Flow-injection analysis was performed to determine the peak area of analyte solutions in 5 mM formic acid in water. (A) (◇) Pure desmetyl raclopride samples were first determined in concentrations between 0.3 and 15 μM and then (■) determination of the same analyte was performed on samples which had been mixed with raclopride in equal concentrations [A] = [B]. (B) Racloprid was then determined by the same procedure by determination of the analyte at (◇) concentrations between 0.3 and 15 μM and then (■) on samples mixed with desmetyl raclopride in equal concentrations [A] = [B].

same sample volume would be injected, in a non-eluting solvent, on to a column with reduced dimensions and a lower flow-rate, an 'on-column focusing' would occur. The concentration in the column would increase and the whole sample would be introduced in the mass spectrometer for analysis. In blood metabolite analysis, the sample amount is limited and it is therefore important to avoid splitting. By use of capillary columns, it should thus be possible to reduce the detection limits more than a 100-fold for the same samples. We are currently working with packed capillary columns to fully utilise this gain in absolute sensitivity.

4. Conclusions

The use of electrospray mass spectrometry in

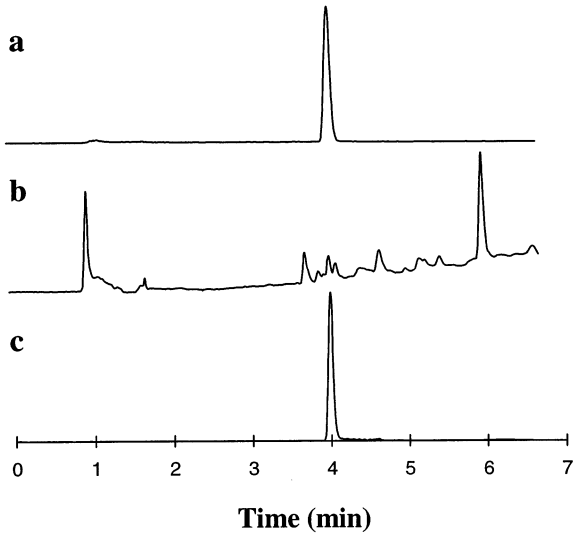

Fig. 3. The synthetic product of ^{11}C-ketamine was analysed with on-line detection of (a) radioactivity, (b) UV absorbance and (c) positive electrospray mass spectrometry with selected ion monitoring of m/z 238 $[M+H]^+$.

the determination of specific radioactivity can provide improved selectivity and sensitivity. In the PET environment, these features will be valuable in method development analysis and in work with purity control and quantification of the final product. With increasing demands on different aspects on work with good manufacturing practice, the mass spectrometer will be a valuable complement to the UV absorbance detector.

The improved detection limits that electrospray mass spectrometry offers has also convinced us to investigate the possibility of performing metabolite analyses on blood samples during a PET investigation. If the sensitivity can be further increased by work with capillary columns, for limited sample volumes, it may be possible to reach the detection limits required for such analysis.

References

[1] H.N. Wagner Jr., Z. Szabo, J.W. Buchanon, Principles of Nuclear Medicine, 2nd ed., W.B. Saunders, Philadelphia, 1995.
[2] A.P. Bruins, Mass Spectrom. Rev. 10 (1991) 53–77.
[3] A.P. Bruins, J. Chromatogr. 554 (1991) 39–46.
[4] A.L. Burlingame, T.A. Baillie, D.H. Russell, Anal. Chem. 64 (1992) 467R–502R.
[5] M.H. Allen, B.I. Shushan, LC-GC 11 (1993) 112–126.
[6] E.C. Huang, T. Wachs, J.J. Conboy, J.D. Henion, Anal. Chem. 62 (1990) 713A–725A.
[7] G.K. Mulholland, M.R. Kilbourn, P. Sherman, J.E. Carey, K.A. Frey, R.A. Koeppe, D.E. Kuhl, Nucl. Med. Biol. 22 (1995) 13–17.
[8] Y. Andersson, N. Tyrefors, S. Sihver, H. Onoe, Y. Watanabe, B. Långström, Doctoral thesis, Acta Universitatis Upsaliensis, No 94, 1995.
[9] B. Långström, H. Lundquist, Radiochem. Radioanal. Lett. 41 (1979) 375–382.
[10] B. Hyllbrant, N. Tyrefors, K.E. Markides, B. Långström, J. Label. Compd. Radiopharm. 37 (1995) 704–705.
[11] E.J.F. Franssen, G. Luurtsema, J. Medema, G.M. Visser, C.M. Jeronismus-Shalingh, A.P. Bruins, W. Vaalburg, Appl. Radiat. Isot. 45 (1994) 937–940.
[12] P.H. Elsinga, A. Van Waarde, G.M. Visser, W. Vaalburg, Nucl. Med. Biol. 21 (1994) 211–217.
[13] J.D. Gilbert, T.V. Olah, D.A. McLoughlin, Am. Chem. Soc. Symp. Ser. 619 (1996) 330–350.
[14] F.E. Kuhlmann, A. Apffel, S.M. Fischer, G. Goldberg, P.C. Goodley, J. Am. Soc. Mass Spectrom. 6 (1995) 1221–1225.
[15] L. Farde, S. Pauli, H. Hall, L. Eriksson, C. Halldin, T. Högberg, L. Nilsson, I. Sjögren, S. Stone-Elander, Psychopharmacology 94 (1988) 471–478.
[16] R.R. MacGregor, J.S. Fowler, A.P. Wolf, C. Halldin, B. Långström, J. Label. Compd. Radiopharm. 25 (1988) 1–9.
[17] J. Eshraghi, S.K. Chowdhury, Anal. Chem. 65 (1993) 3528–3533.
[18] N. Tyrefors, B. Hyllbrant, L. Ekman, M. Johansson, B. Långström, J. Chromatogr. 729 (1996) 279–285.
[19] H.D. Burns, R.F. Dannals, B. Långström, H.T. Ravert, S.E. Zemyan, T. Duelfer, D.F. Wong, J.J. Frost, M.J. Kuhar, H.N. Wagner, J. Nucl. Med. 25 (1984) 1222–1227.
[20] P. Hartvig, J. Valtysson, G. Antoni, G. Westerberg, B. Långström, E. Ratti-Moberg, I. Oye, Nucl. Med. Biol. 21 (1994) 927–934.
[21] C. Halldin, S. Stone-Elander, L. Farde, E. Ehrin, K.J. Fasth, B. Långström, G. Sedvall, Appl. Radiat. Isot. 37 (1986) 1039–1043.
[22] A. Nordberg, P. Hartvig, H. Lundquist, G. Antoni, J. Ulin, B. Långström., J. Neural Transm. 1 (1989) 195–205.
[23] C. Halldin, L. Farde, T. Högberg, N. Mohell, H. Hall, T. Suhara, P. Karlsson, Y. Nakashima, C. Swahn, J. Nucl. Med. 36 (1995) 1275–1281.
[24] M. Bergström, G. Westerberg, T. Kihlberg, B. Långström, Nucl. Med. Biol. 24 (1997) 381–388.
[25] A. Persson, E. Ehrin, L. Eriksson, L. Farde, C. Hedström, J. Litton, P. Mindus, G. Sedwall, J. Psychiatric Res. 19 (1985) 609–622.
[26] P. Kebarle, L. Tang, Anal. Chem. 65 (1993) 972A–986A.
[27] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 70 (1998) 882–889.
[28] M.G. Ikonomou, A.T. Blades, P. Kebarle, Anal. Chem. 62 (1990) 957–967.
[29] D.R. Zook, A.P. Bruins, Int. J. Mass Spectrom. Ion Process. 162 (1997) 129–147.
[30] G. Hopfgartner, K. Bean, J. Henion, R. Henry, J. Chromatogr. 647 (1993) 51–61.