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Quantitative determination of L-775,606, a selective 5-hydroxytryptamine 1D agonist, in rat plasma using automated sample preparation and detection by liquid chromatography–tandem mass spectrometry

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Abstract

L-775,606 is under investigation as a selective 5-hydroxytryptamine 1D agonist for the treatment of migraine. A reliable and sensitive method for the analysis of L-775,606 in plasma was required in order to support preclinical evaluation of this compound. A semi-automated sample preparation method using the Beckman Biomek 2000 workstation to perform all liquid handling tasks has been established. The sample analysis was performed using HPLC–MS–MS with a cycle time of 3.5 min per sample. Intra- and inter-day assay accuracy and precision are excellent with a calibration range of 1–2000 ng/ml and a reproducible limit of quantification of 1 ng/ml.

Keywords: L-775,606; 5-Hydroxytryptamine; Serotonin

1. Introduction

The neurotransmitter serotonin or 5-hydroxytryptamine (5-HT) has long been believed to be involved in the pathogenesis of migraine headache, a debilitating condition which affects 9–16% of the world population [1,2]. Evidence for the involvement of serotonin comes from observations that in patients suffering from the illness the level of serotonin in platelets falls [3] and urinary excretion of its major metabolite 5-hydroxyindole acetic acid is increased [4]. In addition, it has been shown that administration of serotonin during a migraine can abort the headache [5]. Although the exact mechanism of a migraine is still not fully understood it is thought to be caused by combination of vasodilatation of cerebral blood vessels and activation of the trigeminal nerves which leads to the throbbing head pain [6,7].

The anti-migraine agents Sumatriptan, Rizatriptan and Zolmitriptan act by causing constriction of the blood vessel. These actions are mediated by 5-HT receptors, in particular the 5-$HT_{1D/1B}$ subtypes found in the cranial blood vessels [8]. Although effective in the treatment of migraine, side-effects experienced such as chest pain and dizziness are attributed to the 5-HT_{1B} activity in the peripheral vasculature [9]. Therefore it was predicted that a compound with increased selectivity for the 5-HT_{1D} receptor subtype

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over the 5-HT_{1B} receptor subtype would have a much improved side-effect profile.

It was found that a series of piperazine analogues of Rizatriptan achieved improved selectivity whilst retaining anti-migraine properties [10]. Compounds in which the pendant phenyl ring was fluorinated were found to give an optimal efficacy profile as exemplified by the *meta*-fluoro phenyl analogue L-775,606 (Fig. 1), a compound that was selected for further safety evaluation. In order to support future in vivo studies, in which efficacy and other parameters would be assessed against compound exposure, a reliable and reproducible analytical method was sought. Furthermore, with the desire to complete such studies in an optimal timeframe thereby speeding passage to market, a higher-throughput analytical system was sought to overcome the traditional bioanalytical bottleneck. This is caused by the need for lengthy sample clean-up procedures and long analysis times required to remove endogenous components from the sample matrix, such as peptides or protein fragments, that may otherwise interfere with poorly selective analytical techniques such as liquid chromatography (LC)–UV. Tandem mass spectrometry (MS–MS) has recently become the mainstay of bioanalysis due to its high sensitivity and enhanced selectivity for analytes over endogenous matrix components [11,12] and consequently may be used to routinely achieve run times of 5 min or less. This switches the focus onto sample preparation as the critical step in the analytical procedure.

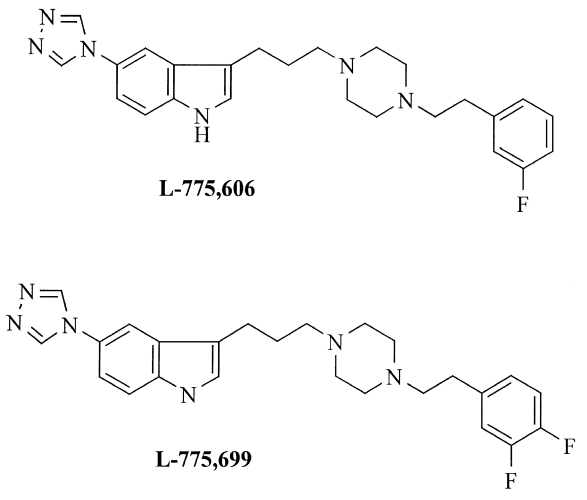

Fig. 1. Structures of L-775,606 and the internal standard L-775,699.

Many laboratories have reported improvements in sample preparation throughput by employing automated techniques for solid-phase extraction (SPE) [13–15] and liquid–liquid extraction [16]. Alternatively, plasma may be injected directly using on-line SPE [17–20] or turbulent flow chromatography [21–23]. A common feature of many solutions is the use of 96-well plate technology in which the wells are conveniently grouped together allowing parallel processing of samples with robotic equipment [24]. We have utilised such an approach to develop a generic protein precipitation assay in a 96-well microtitre plate format with LC–MS–MS detection. All liquid handling steps including preparation of calibration standards, quality control samples, addition of internal standard, transfer of plasma samples and reagent are performed using a Beckman Biomek 2000 robotised workstation with minimal manual intervention. This method has previously been shown to give good accuracy and precision compared to manual methodologies and to give significant savings in time [25]. Furthermore, the use of flow diversion for mass spectrometry, in which the flow for the first minute of every injection is diverted to waste, is utilised and this has previously been demonstrated for a model system [25]. We demonstrate that, for the analysis of L-775,606 the assay is both accurate and precise and affords a significant time saving over preparing samples manually. The validation data for the assay of L-775,606 from rat plasma, which may be used support preclinical studies, is reported.

2. Experimental

2.1. Instrumentation

All liquid handling steps were performed with a Beckman Biomek 2000 workstation (Beckman Coulter, Fullerton, CA, USA) equipped with the following single- and eight-channel pipette tools; P1000L, P200L, P20, MP200 and MP20. The workstation is controlled through Bioworks software version 3.0 running on Windows NT.

Samples in microtitre plates were vortex-mixed using a SMI Multitube Vortexer (Alpha Laboratories, Eastleigh, UK) and centrifuged in a Sorvall RT6000B (DuPont, Stevenage, UK). Microtitre plates were sealed using a Packard Micromate 496 with Topseal-S seals (Packard Biosciences, Pangbourne, UK) and foil seals (Anachem, Luton, UK).

Sample analysis was conducted by LC–MS–MS using a Sciex API 365 mass spectrometer with a Turboionspray interface (PE-Sciex, Toronto, Canada). The HPLC system consisted of an HP1100 binary pumping system with vacuum degassing (Hewlett-Packard, Palo Alto, CA, USA), a HTS-PAL autosampler (Presearch, Hitchin, UK) set up with a 100-μl syringe and divert valve (Jones Chromatography, Hengoed, UK). A Gilson 306 (Anachem) was used for MS make up flow. The HPLC column was a Kromasil KR100 5C8; 5 μm, 50×3.2 mm I.D. (Hichrom, Reading, UK).

2.2. Materials

L-775,606 and L-775,699 (Fig. 1) were synthesised in the laboratory at Merck Research Laboratories (Terlings Park, UK). Control rat plasma was obtained by centrifugation of blood taken from male Sprague–Dawley rats. Conical bottom 96-well polystyrene microtitre plates used for sample preparation (Seco-Well; Fisher, Loughborough, UK). Polypropylene 2-ml square well 96-well plates used for the preparation of all standard solution dilutions (Porvair Sciences, Shepperton, UK). Pipette tips for the Biomek workstation were 1000, 250 and 20 μl (Beckman, High Wycombe, UK). Dimethyl sulfoxide was of AnaLar grade (BDH, Poole, UK), acetonitrile was of HPLC grade (BDH), ammonium formate was AR grade (Fisher, Loughborough, UK) and formic acid was HPLC grade (Romil, Cambridge, UK).

2.3. Instrument conditions

HPLC was performed under isocratic conditions; flow-rate of 0.4 ml/min at ambient temperature. The mobile phase consisted of acetonitrile–25 m*M* ammonium formate buffer adjusted to pH 3.0 with formic acid (30:70, v/v). The injection volume for all samples was 50 μl with post-injection valve washes of 3×100 μl 0.1% formic acid in acetonitrile and 2×100 μl ammonium formate (25 m*M*, adjusted to pH 3.0 with formic acid)–acetonitrile (1:1, v/v). The total cycle time for each sample was 3.5 min.

The LC flow from first 1.5 min of each analysis was diverted, via the switching valve to waste. During this time, a make-up flow of mobile phase (1:1 mixture) was introduced into the mass spectrometer by the Gilson pump. The positions of the switching valve and LC flow during each run is shown in Fig. 2.

Electrospray ionisation was performed in the positive ion mode using nitrogen as the nebulizer, curtain and heater gas at flow-rates of 1.48, 1.44 and 8.0 l/min, respectively. The optimum voltages for orifice (OR) and focusing ring (RNG) were 40 and 210 V, respectively. The turboionspray source was operated at temperature 450°C and ionspray voltage of 5000 V. Analysis of L-775,606 and L-775,699 was achieved in the multiple reaction monitoring (MRM) mode with a collision energy of 35 eV and with nitrogen as the collision gas ($2 \cdot 10^{-5}$ Torr; 1 Torr = 133.322 Pa). The product ion spectra of L-775,606 and L-775,699 under these ionisation conditions with the postulated fragment ions are shown in Fig. 3. Transitions selected from these spectra for quantitative monitoring were 433.3 to 406.0 (L-775,606) and 451.4 to 409.2 (L-775,699).

Data were collected and quantitative processing was performed using PE-Sciex software, Sample Control (version 1.4) and MacQuan (Version 1.6).

2.4. Assay procedure

Stock solutions of L-775,606 for calibration standards and quality control samples were prepared from individual weighings of compound and dissolved in dimethyl sulfoxide to a final concentration of 1000 μg/ml (as base). Similarly a stock solution of L-775,699 (internal standard) was prepared at 1000 μg/ml (as base) in dimethyl sulfoxide. A 0.5-ml volume of each of these solutions were placed into wells A1, C1 and E1 (for calibrant, QC and internal standard, respectively) of a 2-ml polypropylene 96-well plate (the dilution plate – Fig. 4a) and serial dilutions prepared in dimethyl sulfoxide using the Biomek workstation; set up as shown in Fig. 5. Dilutions were prepared in volumes of 1 ml

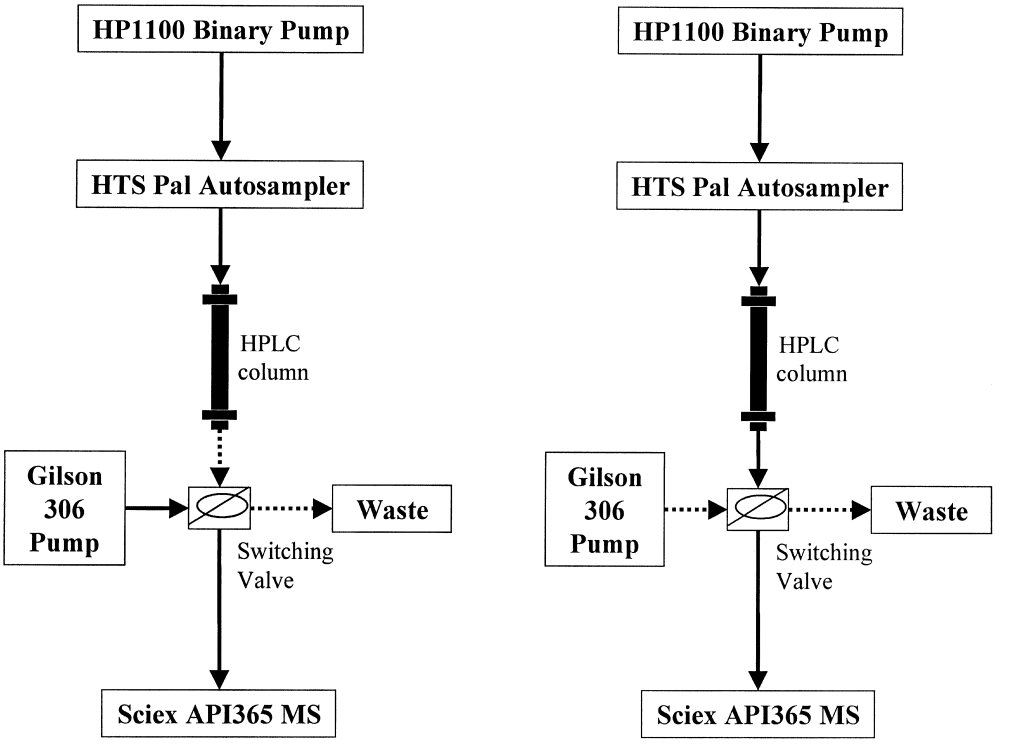

Fig. 2. Positions of the column switching valve and direction of HPLC flow during the 3.5-min cycle of each sample run.

as either 1:2 or 1:10 dilutions with repeated aspiration and dispensing of the liquid to ensure thorough mixing. From these diluted standard solutions volumes of 10 μl were transferred onto a conical bottom 96-well plate (the analysis plate – Fig. 4b) to construct duplicate calibration curves in the range 1 to 2000 ng/ml in plasma. In addition six sets of quality control samples of final concentration 5, 20 and 200 ng/ml were prepared by transferring 10 μl of 0.025, 0.1 and 1 μg/ml solutions of L-775,606 onto the analysis plate. Internal standard was diluted to a concentration of 1 μg/ml of which 10 μl was added to each well of the analysis plate to give a final concentration of 200 ng/ml in plasma.

Control rat plasma was prepared by centrifugation (2000 *g*, 30 min) from blood collected into heparinised tubes taken from male Sprague–Dawley rats and subsequently stored at −80°C until required. After thawing plasma was centrifuged to remove any fibrinogen precipitates and then two 1.8-ml aliquots were transferred to wells H1 and H2 in the dilution plate. The plasma was the transferred by the Biomek, 50-μl aliquots, from the dilution plate to the analysis plate for the preparation of calibration standards and quality control samples. Acetonitrile (100 μl) was then added to all wells.

At this point the automated method was paused and the analysis plate manually removed, foil sealed, vortex-mixed at maximum speed (20s) and centrifuged (15 min, 2000 *g*) to precipitate and pellet plasma proteins. Following centrifugation the seal was removed and the plate replaced on the Biomek worksurface. The programme was then restarted and 50 μl ammonium formate buffer (25 m*M*, pH 3) was

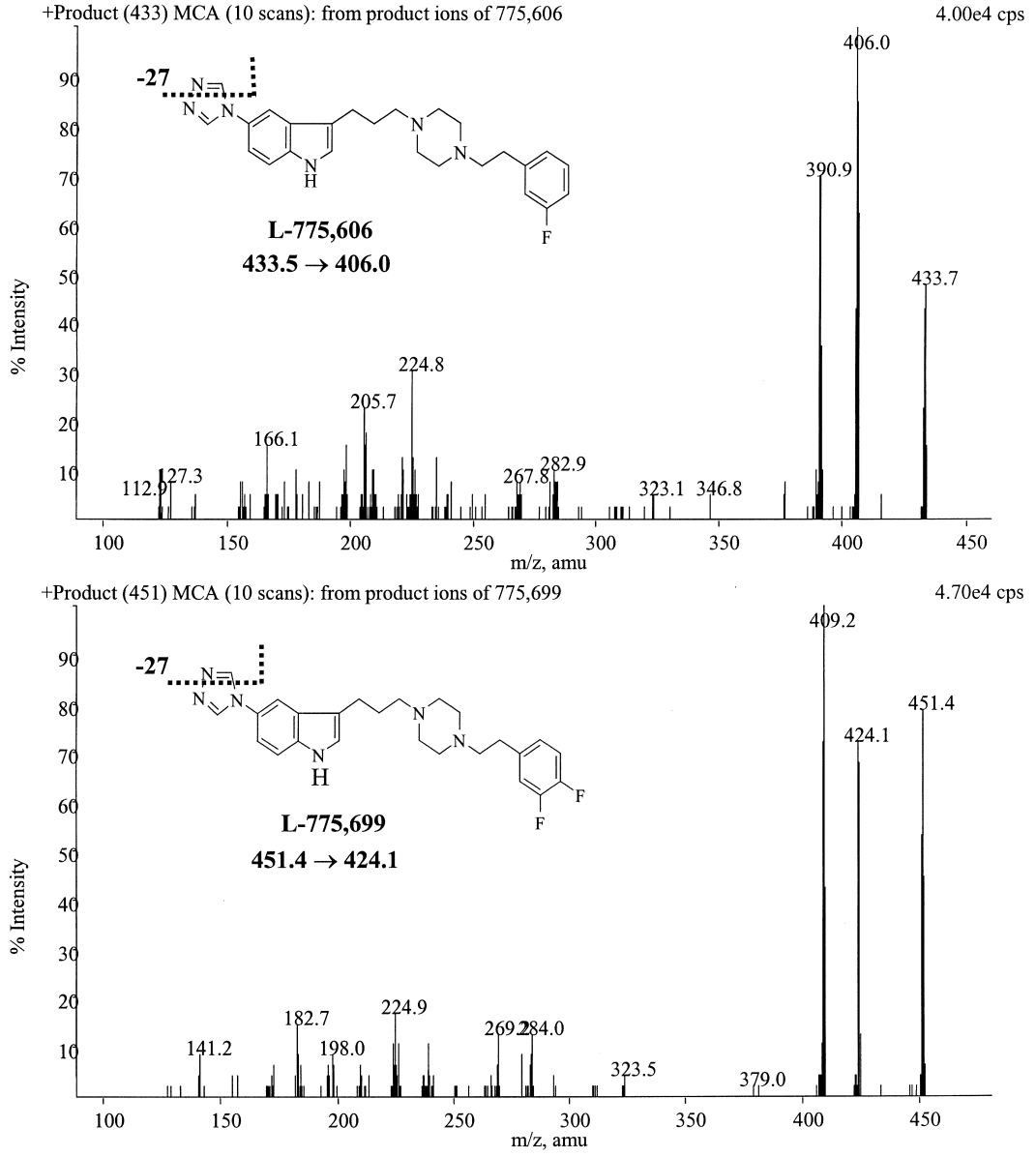

Fig. 3. Product ion mass spectra of (a) L-775,606 and (b) L-775,699.

added to an empty polystyrene conical bottom plate and 100 μl of the supernatant transferred from the protein precipitated samples to the new plate using an eight-channel pipette tool. This plate was then removed, resealed and transferred to the LC–MS–MS system for analysis.

2.5. Assay validation

2.5.1. Linearity

Linearity was tested over the concentration range 1–2000 ng/ml in rat plasma. Calibration standards were prepared in duplicate from freshly prepared

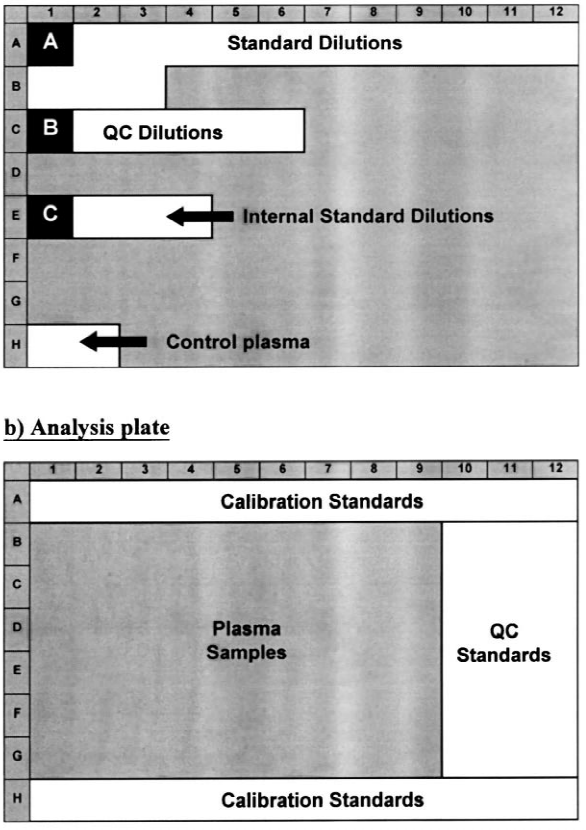

Fig. 4. Layout of (a) dilution plate and (b) analysis plates.

dilutions using the described automated method. Calibrations consisted of 11 concentrations (1, 2, 5, 10, 20, 50, 100, 200, 500, 1000 and 2000 ng/ml in plasma) plus a plasma blank. Comparison of the slope, intercept and correlation coefficients was made for duplicate calibration curves prepared from fresh dilutions of analyte and internal standard stock solutions on six separate occasions.

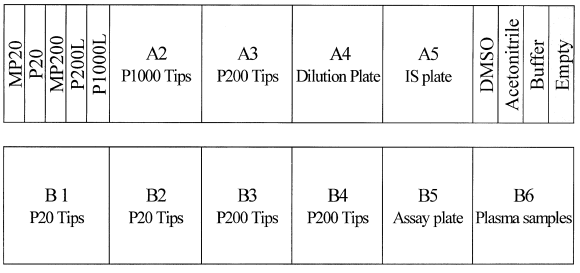

Fig. 5. Layout of the Biomek workstation for plasma sample preparation.

2.5.2. Intra-day accuracy and precision

Intra-day accuracy and precision was tested by preparation of six replicate standards at each point on the calibration line in a 96-well plate using the automated method. A duplicate calibration curve was also constructed and concentrations of the replicate standards were determined. Accuracy and precision of the measured concentrations was calculated for each concentration. In addition the limit of quantification was determined as the lowest point on the calibration curve with accuracy and precision within 20%.

2.5.3. Inter-day accuracy and precision

Inter-day accuracy and precision was tested by comparison of the accuracy and precision of plasma which had been spiked with L-775,606 at concentrations of 5, 20 and 200 ng/ml ($n=6$ per concentration) on 6 separate days. In each case the samples were analysed against a freshly prepared duplicate calibration curve constructed from fresh dilutions of L-775,606 and L-775,699.

2.5.4. Recovery

Recovery of the sample preparation method was determined by comparison of the mass spectrometric response obtained for plasma samples following preparation as described with that obtained for aqueous standards prepared at the same concentrations. Aliquots of plasma were spiked with L-775,606 at 5, 20 and 200 ng/ml on a 96-well plate and protein precipitated using the automated method ($n=6$ per concentration). Aqueous standards were prepared by manually spiking L-775,606 into acetonitrile–ammonium formate (25 m*M*, pH 3) (1:1, v/v) to give the same concentrations.

2.5.5. Specificity

Specificity of the assay was demonstrated by comparison of chromatograms obtained for spiked plasma to blank matrix over a number of injections.

2.5.6. Short-term stability

Short-term stability of L-775,606 in plasma was demonstrated over three freeze–thaw cycles. Three 2-ml aliquots of control plasma were spiked with L-775,606 to give final concentrations of 5, 20 and 200 ng/ml. Three-hundred-μl aliquots from each of these samples were dispensed into Eppendorf tubes ($n=6$ per concentration). One 50-μl aliquot was removed from each of the Eppendorfs and analysed immediately using the described method. Half of the Eppendorfs were then stored at −20°C and the remainder stored at −80°C after rapid freezing on dry ice. These were defrosted and refrozen three times and 50-μl aliquots taken from each sample at each defrost. An hour was left between each freeze–thaw cycle. The samples taken from these aliquots were analysed to determine stability of L-775,606 in plasma after repeated freezing and thawing.

2.5.7. Long-term stability

Long-term stability was determined over 16 weeks. Three aliquots, each containing 6 ml of control plasma were spiked with L-775,606 to give final concentrations of 5, 20 and 200 ng/ml. Aliquots of 50 μl from each sample were dispensed into 96-well conical bottom plates ($n=6$ per concentration per plate to give a total of 11 plates). Six plates were stored frozen at −20°C and six were stored frozen at −80°C. The remaining plate was analysed immediately to determine the initial concentration of L-775,606. Plates from both storage conditions were analysed after 1, 3, 8, 12 and 16 weeks to determine long-term stability of L-775,606 in plasma.

Table 1
Comparison of slopes and intercepts of L-775,606 calibrations in rat plasma

Day	Slope	Intercept	Correlation coefficient
1	0.00523	0.00012	0.9983
2	0.00519	0.0024	0.9987
3	0.00492	0.00215	0.9992
4	0.00506	0.00342	0.9972
5	0.00492	0.00218	0.9989
6	0.00494	0.0045	0.9985
Mean	0.00504	0.00246	0.9985
SD	0.00014	0.00147	0.00069

3. Results and discussion

We have developed a semi-automated sample preparation method for the quantitative analysis of the $5HT_{1D}$ agonist L-775,606 in rat plasma by LC–MS–MS with a run time of 3.5 min per sample. The sample preparation time was 2 h for a full 96-well plate including preparation of all calibration standards and quality controls with minimal manual intervention. In order to run the assay the analyst is required only to supply a deep well plate containing solutions of the calibration and QC standard, internal standard and control plasma. Additionally the Beckman Biomek 2000 is supplied with the required tips, plates and reagents prior to starting the method. The only manual intervention then required is off-line plate sealing, vortex mixing and centrifugation. It is anticipated that in future it will be possible to integrate suitable devices to perform these steps on-line and therefore provide a fully automated procedure.

This methodology offers a major time saving compared with manual sample preparation, which would typically take one analyst 1 whole day for the same number of samples. This frees the analyst for other activities such as LC–MS–MS optimisation, data processing, etc. In addition since this assay takes approximately 2 h to run it is possible to prepare three such plates of samples (approximately 300 samples) for more efficient use of the mass spectrometer during the overnight period. Using this methodology it is therefore possible to analyse approximately 1500 samples per week. This increase in throughput allows results from bioanalysis to be provided in a more timely manner and have an impact on the drug discovery process at a much earlier stage.

Furthermore, the use of flow diversion is important to preserve the mass spectrometer source from contamination, which could potentially reduce instrument performance.

Validation of this methodology, summarised below has demonstrated the assay to have excellent intra- and inter-day accuracy and precision that are well within acceptance criteria of US Food and Drug Administration (FDA) guidelines. In addition we have demonstrated that L-775,606 shows short- and long-term stability in rat plasma.

3.1. Linearity, specificity and intra- and inter-day results

The linearity and reproducibility of calibration curves prepared for L-775,606 in rat plasma using the method described in Section 2.4 was compared on six separate occasions. Curves were fitted with a linear regression line ($y=mx+c$) and weighted $1/x$. The slope, intercept and correlation coefficients obtained on these six separate occasions are shown in Table 1. The curves showed good linearity between 1 and 2000 ng/ml with a typical R^2 value of >0.997. Reproducibility of the calibration slopes was good with average±standard deviation (SD), 0.00504 ± 0.00014 ($n=6$). Intercept values were low, typically <0.002.

To demonstrate specificity of the method chromatograms of blank extracted plasma were compared

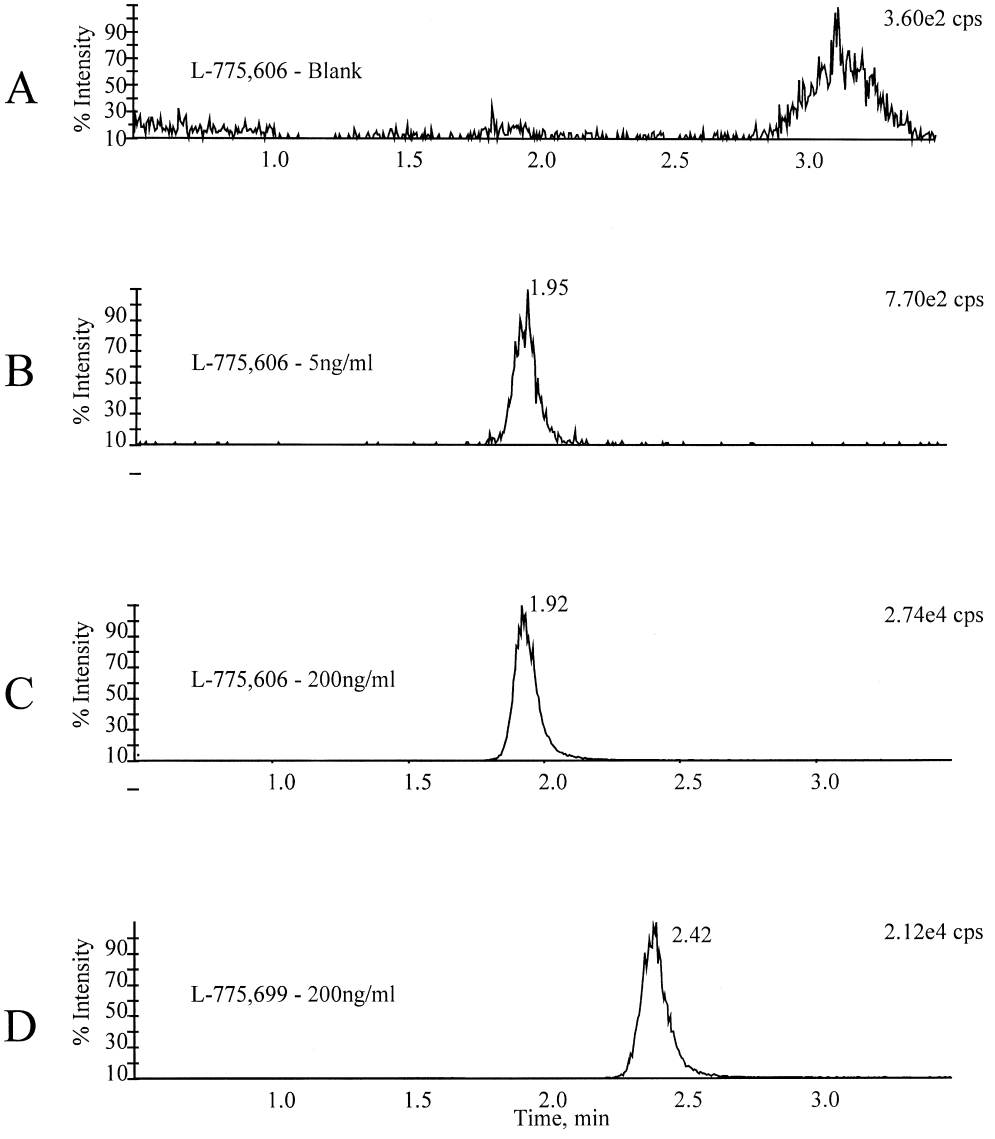

Fig. 6. Typical chromatograms showing (A) blank plasma, (B) 5 ng/ml L-755,606, (C) 200 ng/ml L-775,606 and (D) 200 ng/ml L-775,606.

Table 2
Intra-day accuracy and precision for the determination of L-775,606 in plasma

Target concentration (ng/ml)	*n*	Mean measured concentration (ng/ml)	Precision (%)	Accuracy (%)
1	6	1.1	7	109
2	6	2.0	4	99
5	6	4.9	5	97
10	6	10	2	103
20	6	20	3	98
50	6	52	2	105
100	6	104	3	104
200	6	204	4	102
500	6	521	1	104
1000	6	988	2	99
2000	6	1935	1	97

with spiked plasma. Typical chromatograms for blank plasma and spiked plasma at concentrations of 5 and 200 ng/ml L-775,606 along with a chromatogram showing 200 ng/ml internal standard are shown in Fig. 6. Blank chromatograms were free from any interference at the retention time of the analyte.

Intra-assay accuracy and precision data, compared across the whole calibration range ($n=6$) are shown in Table 2. Mean concentrations measured are reported along with accuracy and precision values calculated from the mean data. Intra-assay accuracy was excellent with values ranging between 97 and 109%, which is within acceptable limits set of 80–120%. Intra-assay precision was also excellent with a value of 7% at 1 ng/ml and <5% at all other concentrations. The limit of quantification of the assay was determined as 1 ng/ml (with accuracy of 109% and precision of 7%).

Inter-day accuracy and precision, compared at three concentrations on six separate occasions is shown in Table 3. The accuracy and precision data calculated from the mean data from all six occasions was calculated and showed good reproducibility on all six occasions with accuracy ranging from 93 to 100% and precision ranging between 5 and 10%.

3.2. Recovery and stability

Recovery of L-775,606 using this method, compared to an aqueous standard at three concentrations is shown in Table 4. The efficiency of the sample preparation method was demonstrated as >80% and showed little variation.

The short- and long-term stability of L-775,606 in rat plasma was demonstrated over 16 weeks at storage temperatures of −20°C and −80°C and during three freeze–thaw cycles (Tables 5 and 6).

Table 3
Inter-day accuracy and precision for the determination of L-775,606 in plasma

Day	*n*	Mean measured concentration (ng/ml)±SD		
		5 ng/ml	20 ng/ml	200 ng/ml
1	6	4.6±0.3	20.2±0.9	201.1±9.8
2	6	4.7±0.3	20.0±0.2	205.6±3.1
3	6	4.7±0.2	21.9±4.0	209.3±4.9
4	6	4.8±0.3	20.0±0.5	202.4±4.1
5	6	4.5±0.3	18.3±0.7	180.5±6.0
6	6	4.6±0.2	19.6±0.4	199.6±6.2
Mean precision (%)		5	10	5
Mean accuracy (%)		93	100	100

Table 4
Recovery of L-775,606 from plasma using automated sample preparation

Sample (ng/ml)	*n*	Mean recovery (%), ±SD
5	6	90±7
20	6	83±4
200	6	89±8

The % change between the initial concentrations and the concentration at week 16 were 6–15% at −20°C and 6–8% at −80°C. These changes are within the acceptable variation of the assay and therefore L-775,606 has been shown to be stable in plasma up to 16 weeks. Similarly when L-775,606 undergoes the freeze–thaw process the percentage difference is very low (4–6% at −20°C and 2–3% at −80°C) demonstrating that L-775,606 is stable to freeze–thaw processes.

4. Conclusions

We have demonstrated a method for the quantitative analysis of L-775,606 in plasma which shows excellent sensitivity and selectivity plus a high degree of accuracy and precision. The linear range of 1–2000 ng/ml and limit of quantification of 1 ng/ml achieved using this method are suitable for the analysis of samples from preclinical studies in rat. A typical plasma concentration versus time profile for male Sprague–Dawley rat after intravenous (3 mg/kg) and oral (3 mg/kg) administration is shown in Fig. 7. This data shows that L-775,606 has a reasonable pharmacokinetic profile in rat with oral bioavailability of 26% and half-life 1.3 h.

The use of semi-automated sample preparation using a Beckman Biomek 2000 robotised system has dramatically increased the throughput of the assay, compared to manual methodologies, allowing the preparation of three 96-well plates per day for

Table 5
Stability of L-775,606 from plasma stored at −20°C and during three freeze–thaw cycles

Nominal concentration (ng/ml)	*n*	Mean measured concentration (ng/ml)±SD					
		Initial	Week 1	Week 3	Week 8	Week 12	Week 16
5	6	5.3±0.4	5.7±0.5	5.6±0.4	5.1± 0.4	5.0±0.7	4.5±0.3
20	6	22.1±1.2	23.2±1.1	21.7±1.5	20.6±1	19.7±1.0	18.8±1.1
200	6	210.5±10.8	221.8±7.2	204.3±13.1	205.3±5.5	183.0±6.8	199.4±7.4
			Freeze–thaw 1	Freeze–thaw 2	Freeze–thaw 3		
5	3	5.7±0.2	5.1±0.2	5.6±0.1	5.5±0.2		
20	3	21.1±0.9	20.4±0.2	20±0.3	19.8±0.6		
200	3	205.4±2.5	204.2±5.8	198.1±3.3	194.9±3.9		

Table 6
Stability of L-775,606 from plasma stored at −80°C and during three freeze–thaw cycles

Nominal concentration (ng/ml)	*n*	Mean measured concentration (ng/ml)±SD					
		Initial	Week 1	Week 3	Week 8	Week 12	Week 16
5	6	5.3±0.4	5.7±0.5	6.1±1.1	4.5±0.6	4.7±1.0	5.0±0.6
20	6	22.1±1.2	22.9±0.7	23.5±0.8	19.1±1.2	17.0±3.6	20.3±2.3
200	6	210.5±10.8	223.6±10.4	243.2±8.38	193.7±11.9	191.7±12.9	198.3±13.7
			Freeze–thaw 1	Freeze–thaw 2	Freeze–thaw 3		
5	3	5.3±0.2	5.5±0.2	5.2±0.3	5.3±0.4		
20	3	20.6±0.04	20.9±0.3	20.6±0.14	20.2±0.4		
200	3	201.0±11.0	199.4±3.1	205.7±6.0	198.9±5.4		

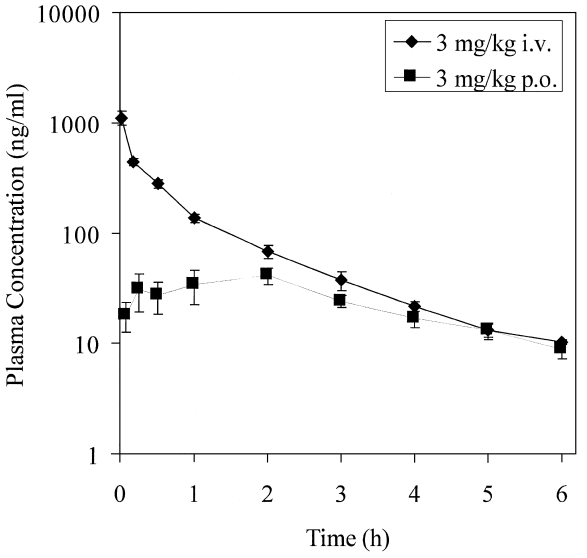

Fig. 7. Plasma concentration profiles of L-775,606 in rats following intravenous administration (3 mg/kg) and oral administration (3 mg/kg).

overnight analysis by mass spectrometry. The use of flow diversion reduces the possibility of MS source contamination from these samples and decreases instrument downtime. Obvious benefits from this work include more efficient use of MS instrumentation and analyst time.

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References

[1] W.F. Stewart, R.B. Lipton, D.D. Celentano, M.L. Reed, JAMA 267 (1992) 64.
[2] M. Goldstein, T.C. Chen, Adv. Neurol. 3 (1982) 377.
[3] D.A. Curran, H. Hinterberger, J.W. Lance, Brain 88 (1965) 997.
[4] F. Sicuteri, A. Testi, B. Anselmi, Int. Arch. Allergy 19 (1961) 55.
[5] R.W. Kimball, A.P. Friedman, E. Vallejo, Neurology 10 (1960) 107.
[6] K.W. Johnson, L.A. Phebus, M.L. Cohen, Prog. Drug Res. 51 (1998) 219.
[7] P.J. Pauwels, G.W. John, Clin. Neuropharmacol. 22 (1999) 123.
[8] P.J. Goadsby, CNS Drugs 10 (1998) 271.
[9] J.P. Ottervanger, T.B. van Witsen, H.A. Valkenburg, D.E. Grobbee, B.H.C. Stricker, Eur. J. Clin. Pharmacol. 47 (1994) 305.
[10] A.M. Macleod, L.J. Street, A.J. Reeve, R.A. Jelley, F. Sternfield, M.S. Beer, J.A. Stanton, A.P. Watt, D. Rathbone, V.G. Matassa, J. Med. Chem. 40 (1997) 3501.
[11] J.D. Gilbert, T.V. Olah, D.A. McLoughlin, ACS Symp. Ser. 619 (1996) 330.
[12] D.M. Dulik, W.H. Schaefer, J. Bordas-Nagy, R.C. Simpson, D.M. Murphy, G.R. Rhodes, Mass Spectrom. Biol. Sci. (1996) 425.
[13] J.P. Allanson, R.A. Biddlecombe, A.E. Jones, S. Pleasance, Rapid Commun. Mass Spectrom. 10 (1996) 811.
[14] A.C. Harrison, D.K. Walker, J. Pharm. Biomed. Anal. 16 (1998) 777.
[15] S. Pleasance, R.A. Biddlecombe, Methodol. Surv. Bioanal. Drugs 25 (1998) 205.
[16] S. Steinborner, J. Henion, Anal. Chem. 71 (1998) 2340.
[17] M. Hedenmo, B.-M. Eriksson, J. Chromatogr. A 661 (1994) 153.
[18] E. Brandsteterova, D. Romanova, D. Kralikova, L. Bozekova, M. Kriska, J. Chromatogr. A 665 (1994) 101.
[19] A. Marchese, C. McHugh, J. Kehler, H.-G. Bi, J. Mass Spectrom. 33 (1998) 1071.
[20] D.A. Mcloughlin, T.V. Olah, J.D. Gilbert, J. Pharm. Biomed. Anal. 15 (1997).
[21] H.M. Quinn, J.J. Takarewski, Jr., PCT Int. Appl. WO 9 716 724 (1997).
[22] J. Ayrton, G.J. Dear, W.J. Leavens, D.N. Mallett, R.S. Plumb, Rapid Commun. Mass Spectrom. 11 (1997) 1953.
[23] J. Ayrton, G.J. Dear, W.J. Leavens, D.N. Mallett, R.S. Plumb, J. Chromatogr. A 828 (1998) 199.
[24] Y.-F. Cheng, U.D. Neue, L. Bean, J. Chromatogr. A 828 (1998) 273.
[25] A.P. Watt, D. Morrison, K.L. Locker, D.C. Evans, Anal. Chem. 72 (2000) 979.