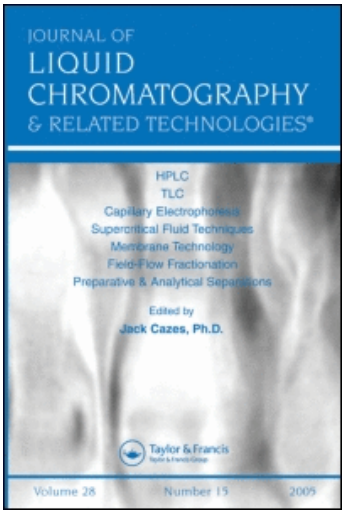

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A HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR THE QUANTITATION OF IMPURITIES IN AN NMDA ANTAGONIST USING EVAPORATIVE LIGHT SCATTERING DETECTION

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ABSTRACT

A reversed-phase high-performance liquid chromatography (HPLC) method utilizing an evaporative light scattering detector (ELSD) was developed for a new NMDA (N-methyl-D-aspartate) antagonist. This method permits quantitation of both the bulk drug substance purity and the related materials possible within the bulk drug substance. The method is compatible with LC/MS and the mass spectral data were obtained for each component in the bulk drug substance.

INTRODUCTION

Pharmaceutical compounds are routinely evaluated for bulk drug substance purity as well as content of possible impurities and degradation products. HPLC is currently the most common technique used to quantitate both bulk drug substance purity and nonvolatile impurities in a drug substance. Typically, HPLC with UV detection is chosen due to high sensitivity, ease of use and linear range.

Complications often arise in quantitation of unknown impurities since many of these entities may lack a UV chromophore or the chromophore may have vastly different properties when compared to the spectral properties of the parent drug substance. The recent introduction of the evaporative light scattering detector (ELSD) into the arena of HPLC detectors helps to resolve this nonequivalence response issue.

The ELSD has been introduced commercially and has gained acceptance as a sensitive universal detector.[1-2] The ELSD operates by nebulizing the volatile effluent from the HPLC column into a fine mist. The mist is then carried through a heated drift tube which evaporates the mobile phase and leaves behind the nonvolatile solute particles. The fine cloud of solute particles is carried at a high speed through a beam of light causing scattering which is detected. The amount of light scattered is dependent upon the size, shape and number of particles and therefore proportional to the concentration.

The ELSD is not influenced by the UV spectral properties of the solvents used for mobile phases, therefore the ELSD is not subject to baseline drift from gradient elution. The choice of acceptable solvents is expanded since the spectral background is not an issue with the ELSD. The ELSD is not affected with sample solvent interferences and the sample response is independent of the chemical structure of the solute. A limitation of the ELSD requires the complete volatilization of all mobile phase components. Addition of nonvolatile components to the mobile phase would cause an elevated background by the continuous generation of solid particles into the light source. The elevated background decreases the sensitivity of the detector for the sample components.

The current chromatographic literature demonstrates the applicability of the ELSD for use with phospholipids,[3-9] triglycerides, fats and fatty acid esters,[10-15] carbohydrates,[16-17] synthetic polymers,[18] steroids[19] and inorganic counter ions.[20] Recently, Lafrosse *et al* demonstrated the applicability of using the ELSD for pharmaceutical compounds.[21] While the HPLC/ELSD combination has been used for quantitation of drug entities, the lack of interest in the use of the technique for determination of impurities and degradation products is surprising. The universal capabilities of this detector lends itself to such uses.

The intent of this paper is to show the applicability of the ELSD to accurately detect and quantitate impurity levels in a pharmaceutical compound. Our laboratory recently developed a method to determine the purity of an NMDA antagonist with the structure shown in Figure 1. This chemical entity has insufficient UV chromophores and therefore a more sensitive means of detection was desired. Various UV and fluorescence derivatization schemes are possible but such manipulation introduces their own source of errors into analytical procedures. Furthermore, the lack of nonaqueous solubility of this NMDA antagonist limits the number of derivatization schemes that are possible. Gas chromatography (GC) could not be used because the compound decomposes at 283°C. This NMDA antagonist presented an ideal situation for employment of the ELSD. The detector technology should provide a means for quantitation of the main compound, as well as offering a means for determination of potential impurities in the drug substance. A high-low approach was used to quantitate impurity levels.[22] Quantitation was based directly on the peak area responses of the substances injected which is a principle of the ELSD, however, it should be noted that differing physical properties in samples (i.e., viscosity, refractive index, temperature sensitivity) can result in a different droplet size and subsequently different response for similar concentrations. The HPLC method for this NMDA antagonist was then run using LC/MS to identify the unknown impurities.

MATERIALS AND METHODS

Chemicals

The NMDA antagonist (Eli Lilly compound LY235959, [(-)-3S,4aR,6S,8aS-6-(phosphonomethyl)-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxylic acid]) was synthesized from m-tyrosine.[23] The bisphosphonic acid and 6-methyl impurities were also prepared within Eli Lilly and Company (Indianapolis, IN). Trifluoroacetic acid (TFA) A.R. was purchased from EM Science (Gibbstown, NJ). The acetonitrile (ACN) was HPLC grade and

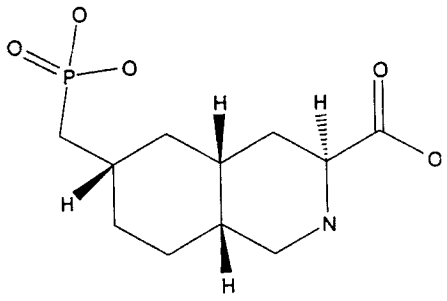

FIGURE 1. Structure of NMDA antagonist.

purchased from Mallinckrodt (Paris, KY). Water was deionized and filtered through a Milli-Q™ water purification system (Millipore, New Bedford, MA). NF grade nitrogen was used for the ELSD.

Apparatus

The HPLC system used for this study consisted of a Hewlett-Packard 1050 autoinjector, pump and UV detector (Wilmington, DE). A Varex IIA ELSD (Burtonsville, MD) was used in tandem with the UV detector set at 205 nm. A Zorbax® SB-Phenyl 250 x 4.6 mm I.D. column (Mac Mod Analytical, Chads Ford, PA) was used to separate the impurities from the NMDA antagonist. A Sciex API III LC/Ionspray Mass Spectrometer (Toronto, Ontario, Canada) was used to determine the mass of the impurities. A scan range of 150 to 600 daltons was used with a scan rate of 2.4 seconds/scan. The ionspray ionization mode was used with a post-column split ratio of 20:1 (20 μL/minute flow to the mass spectrometer source).

Assay and Chromatographic Conditions

A binary reversed-phase gradient system was used to ensure all impurities were eluted from of the column. The gradient profile and mobile phases are described in Table 1. Equilibration time before the next injection was 20 minutes.

TABLE I

Gradient System for the NMDA Antagonist Impurity Assay

Time (min.)	% A[a]	% B[b]
0.0	100	0
12.0	100	0
22.0	0	100
38.0	0	100
40.0	100	0

[a]A = 1% Acetonitrile/99% water pH adjusted to 2.4 with trifluoroacetic acid
[b]B = 60% Acetonitrile/40% water pH adjusted to 2.4 with trifluoroacetic acid

The mobile phase flow rate was set at 0.4 mL/min. The column temperature was ambient and injection volume was 50 µL. Impurity levels in the NMDA antagonist drug substance were assessed using a high-low approach.[22] Sample concentration of 3 mg/mL for the NMDA antagonist was prepared in mobile phase A as the high concentration sample. The sample was placed in an ultrasonic bath for approximately 5 minutes to enhance dissolution. A 1:10 dilution yielded a 0.3 mg/mL solution for the low concentration sample. The low concentration solution was then analyzed by HPLC followed by the high concentration solution.

RESULTS AND DISCUSSION

This method for the determination of impurities in the NMDA antagonist was validated for the parameters of linearity, precision, selectivity and limit of detection. The method uses a high-low chromatography approach to determine impurity levels. High-low chromatography is a sampling technique used to improve the detection limit of trace components in a bulk drug substance by extending the dynamic range of the detection system.[22] Prior to validation, the Varex IIA ELSD was optimized for nitrogen gas flow and temperature. The validation sample lot was then run on an LC/MS system where the impurities were identified.

ELSD Optimization

The ELSD is relatively independent of molecular functional groups within a chemical entity. To illustrate this, a UV detector and an ELSD were connected in series allowing for the superimposition of the two detector responses on a single chromatogram corresponding to the same sample injection. A mixture of a small aliquot of the mother liquor from the NMDA antagonist synthesis combined with 2 mg/mL of the NMDA antagonist bulk drug substance resulted in a situation where the main compound (NMDA antagonist) does not have a suitable chromophore and some of the impurities do (Figure 2). In this situation, the ELSD proves to be a more suitable detector than UV because the ELSD response is independent of the molecular function groups.

Optimization of the Varex IIA ELSD is critical for an impurity assay because sensitivity levels can be dramatically reduced under sub-optimal conditions. Operating conditions for the Varex IIA ELSD were optimized to obtain the greatest signal-to-noise ratio by controlling both the nitrogen gas flowing into the nebulizer and the temperature of the drift tube. The nitrogen gas flow rate into the nebulizer controls the droplet size which is critical for obtaining efficient vaporization of the mobile phase and maximum sensitivity of the detector. A nitrogen gas flow of 3.0 L/min was used for the validation. Figure 3 illustrates the effect of increasing nitrogen gas flow into the nebulizer on the NMDA antagonist peak signal-to-noise ratio. It is important to note that changing parameters in the HPLC assay would require reoptimization, however, maintaining the established validated HPLC conditions did not require recalibration of the detector over time.

It is necessary to completely evaporate the mobile phase prior to detection by the laser for maximum sensitivity. Figure 4 shows the effect of increasing the drift tube temperature on the NMDA antagonist peak signal-to-noise ratio. The optimum temperature of the drift tube used for this validation was 150°C. The effect on peak response caused by temperature adjustments to the drift tube is minor compared to changes in the nitrogen gas flow rate into the nebulizer.

Linearity

It is usual practice to perform linearity determinations over a wide range of sample concentrations to fully assess the linear dynamic range of the detection

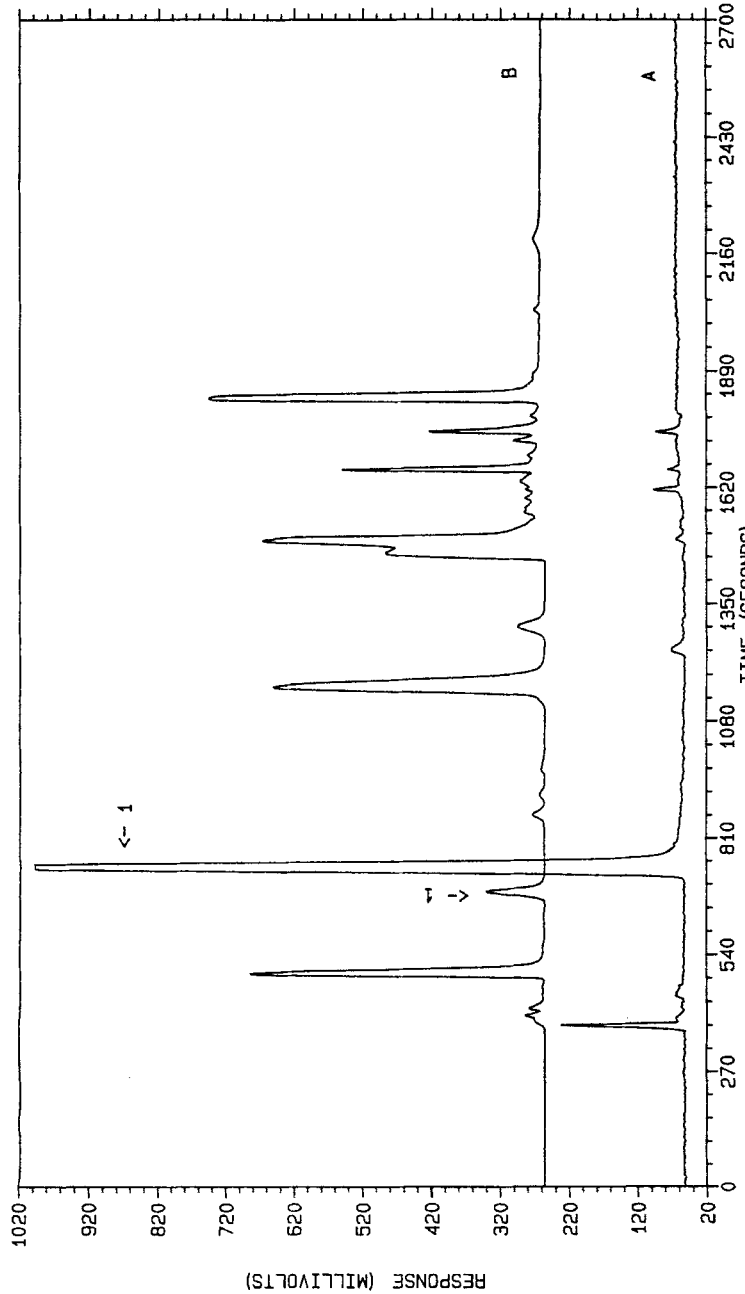

FIGURE 2. Sample injection of a mixture of a small aliquot of the mother liquor from the NMDA antagonist synthesis combined with 2 mg/mL of the NMDA antagonist as detected by ELSD (A) and UV (B). Peak 1 = NMDA antagonist drug substance.

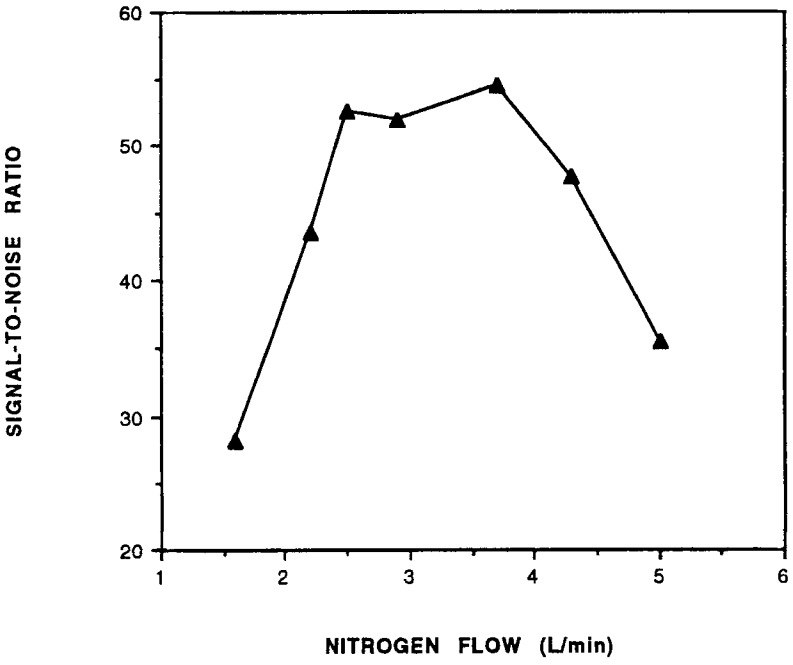

FIGURE 3. The effect of increasing nitrogen gas flow into the nebulizer on the NMDA antagonist peak signal-to-noise ratio.

system. Since a high-low approach is used, the linearity of the impurities in the high concentration sample and linearity of the NMDA antagonist in the low concentration sample were evaluated separately. The NMDA antagonist peak is off-scale for the high concentration samples and therefore cannot be evaluated. The three impurities detected in the sample lot were evaluated using six sample preparations over a concentration range of 1.0-4.3 mg/mL of the NMDA antagonist to establish the system linearity. These impurities peak areas were found to be linear with respect to increasing concentration. The corresponding correlation coefficients for peaks 1-3 (Figure 5) were 0.9997, 0.9938 and 0.9940, respectively.

The linearity of the NMDA antagonist peak was determined with the low sample concentration by injection of six samples representing a concentration range

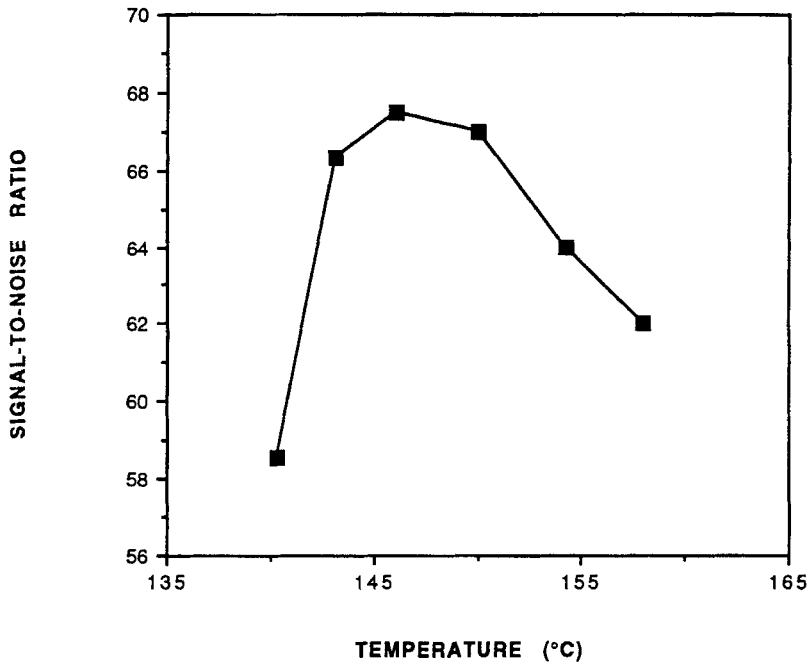

FIGURE 4. The effect of increasing the drift tube temperature on the NMDA antagonist peak signal-to-noise ratio.

of 0.10-0.43 mg/mL of the NMDA antagonist which corresponds to a 1:10 dilution of the high concentration samples. The NMDA antagonist peak areas were found to be linear with respect to increasing concentration. The resulting correlation coefficient was 0.9987.

Precision

The precision of the method was evaluated by injecting singly five *individually* prepared high concentration samples and the corresponding five low sample concentrations. The total impurity average for the five samples was determined to be 5.6% with an relative standard deviation of 1.3%. Figure 5 illustrates a typical sample chromatogram.

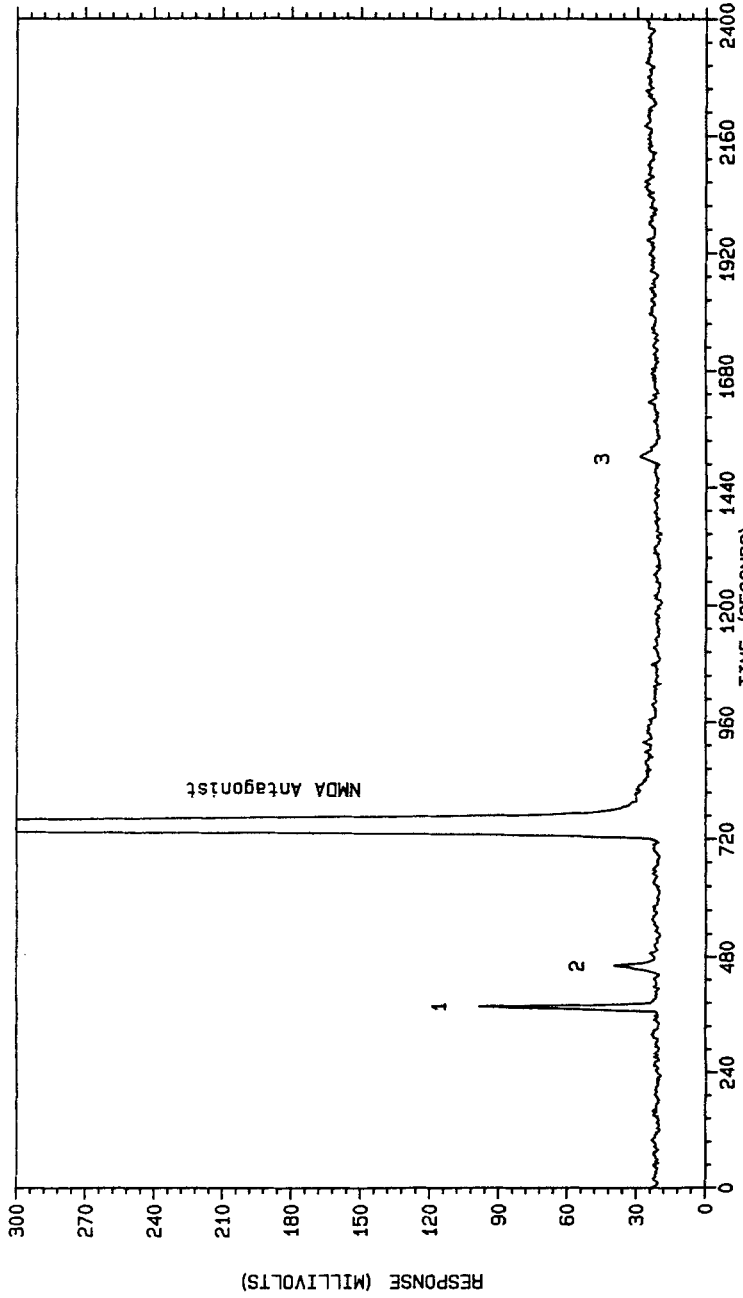

FIGURE 5. Sample chromatogram of NMDA antagonist drug substance high concentration prepared at 3 mg/mL. Peak 1 = bisphosphonic acid, peak 2 = unknown impurity, peak 3 = 6-methyl impurity. The NMDA antagonist peak is off-scale.

Selectivity

As part of the United States Pharmacopeia (USP) guidelines for validation, a method must be shown to have selectivity for the analyte of interest. For this method, selectivity was demonstrated by showing separation of all available impurities including starting materials, process intermediates and degradation products from the NMDA antagonist.

Limit of Detection (LOD)

The LOD is defined as the lowest concentration of a sample that can be clearly detected above baseline noise. The LOD was experimentally determined by making serial dilutions of each compound and then analyzing by HPLC. The LOD was determined to be 0.5 micrograms injected on column for the NMDA antagonist, 0.5 micrograms for bisphosphonic acid and 0.6 micrograms for the 6-methyl impurity. To maintain the LOD, the nebulizer must be cleaned periodically. Increased baseline noise, increased pressure reading and decreased peak response are indicators that the nebulizer is becoming contaminated.

Identification of Impurities

The LC/MS analyses were conducted using chromatographic conditions of the HPLC/ELSD experiments. Analyses were accomplished using a freshly prepared 20 μL injection volume of a 10 mg/mL solution of the NMDA antagonist. The three impurity peaks identified numerically and the NMDA antagonist peak in Figure 5 corresponded to the peaks observed with the LC/MS system.

Peak 1 corresponded very closely to the void volume and contained a variety of ions. The most prominent signal had an m/z of 176.9. Tetraethyl methylene bisphosphonate is used for a reaction in the synthesis. A by-product of this reaction would be bisphosphonic acid (see Figure 6) which has an m/z of 176.9 daltons. The retention time of the peak 1 impurity was then matched with an authentic sample of bisphosphonic acid using the HPLC/ELSD system. Bisphosphonic acid was also observed by ^{31}P-NMR in the NMDA antagonist sample validation lot. It is interesting to note that bisphosphonic acid was the major

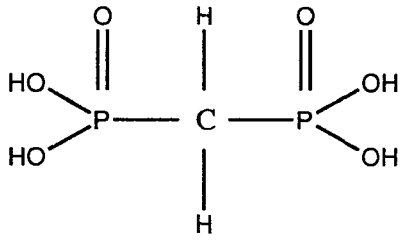

FIGURE 6. Structure of bisphosphonic acid.

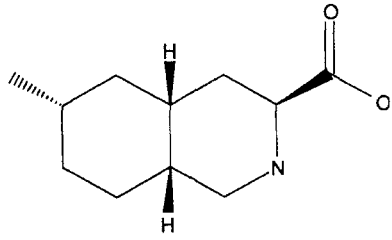

FIGURE 7. Structure of 6-methyl impurity.

impurity found in the validation lot yet it provided no UV response, further illustrating detection with UV could miss a source of impurity in a pharmaceutical compound.

Peak 2 occurred just after the void volume and contained primarily a component having m/z of 196.0. The structure of this impurity has not yet been confirmed.

Peak 3 is detected after completion of the gradient and yielded a very clean spectrum with a m/z of 198.2. This corresponded to the 6-methyl impurity (MW 197.3) which is a possible synthesis contaminant carried through from a previous step in the synthesis. The structure of the 6-methyl impurity is shown in Figure 7. An authentic sample of the 6-methyl impurity matched the retention time of that in the validation sample lot using the HPLC/ELSD system.

The NMDA antagonist (MW 277.3) peak provided the most intense signal and resulted in the expected m/z 278.3 ion (MH+). Another component with m/z of 554.9 was also observed. This signal was attributed to a gas phase dimer (M2H+) of the NMDA antagonist.

CONCLUSION

The applicability of a commercially available evaporative light scattering detector for the determination of impurities in a pharmaceutical compound has been demonstrated. The validation of this assay shows that the ELSD is an effective and practical alternative to conventional detectors. Linearity, precision, sensitivity and compatibility with HPLC have been shown to be more than adequate. Since the ELSD is capable of detecting many types of solutes regardless of functional groups, the versatility of an ELSD provides a practical tool for the analytical chemist.

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REFERENCES

1. J. M. Charlesworth, Anal. Chem., 50:1414 (1978)
2. A. Stolyhwo, H. Colin, G. Guiochon, J. Chromatogr., 265:1 (1983)
3. H. A. Norman, J. B. St. John, J. Lipid Res., 27:1104, (1986)
4. M. D. Grieser, J. N. Geske, J. Am. Oil Chem. Soc., 66:1484 (1989)
5. W. W. Christie, J. Lipid Res., 26:507 (1985)

6. L. Breton, B. Serkiz, J-P. Volland, J. Lepagnol, J. Chromatogr., 497:243 (1989)

7. T. L. Mounts, S. L. Abidi, K. A. Rennick, J. Am. Oil Chem. Soc., 69:438 (1992)

8. S. L. Melton, J. Am. Oil Chem. Soc., 69:784 (1992)

9. W. S. Letter, J. Liq. Chromatogr., 15(2):253 (1992)

10. A. Stolyhwo, H. Colin, G. Guiochon, Anal. Chem., 57:1342 (1985)

11. H. C. Gerard, R. A. Moreau, W. F. Fett, S. F. Osman, J. Am. Oil Chem. Soc., 69:301 (1992)

12. A. Stolyhwo, M. Martin, G. Guiochon, J. Liq. Chromatogr., 10:1237 (1987)

13. J. L. Perrin, A. Prevot, A. Stolywho, G. Guiochon, Revue Francaise des Corps Gras, 12:495 (1984)

14. W. S. Letter, J. Liq. Chromatogr., 16(1):225 (1992)

15. J.C. Robinson, R. Macrae, J. Chromatogr., 303:386 (1984)

16. R. Macrae, J. Dick, J. Chromatogr., 210:138 (1981)

17. R. Macrae, L. C. Trugo, J. Dick, Chromatographia, 15:476 (1982)

18. T. H. Mourey, J. Chromatogr., 357:101 (1986)

19. P. A. Asmus, J. B. Landis, J. Chromatogr., 316:461 (1984)

20. J. A. Peterson, D. S. Risley, J. Liq. Chromatogr., 18(2):331 (1995)

21. M. Lafosse, C. Elfakir, L. Morin-Allory, M. Dreux, J. High Res. Chrom., 15:312 (1992)

22. E. L. Inman, H. J. Tenbarge, J. Chromatographic Sci., 26:89 (1988)

23. P. L. Ornstein, N. K. Augenstein, M. B. Arnold, J. Org. Chem., 59:7862 (1994)

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