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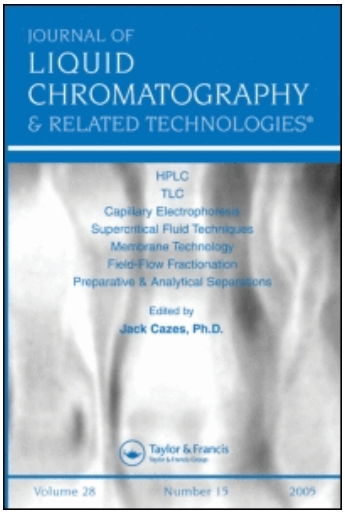

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Validated HPLC Method for the Quantitative Analysis of a 4-Methanesulfonyl-Piperidine Hydrochloride Salt

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Abstract: An isocratic reversed phase high performance liquid chromatographic method with charged aerosol detection (Corona™ CAD™) was developed and validated for the quantitative determination (assay) of 4-methanesulfonyl-piperidine (MSP). This compound has no ultraviolet (UV) chromophore and showed no retention on a reversed phase column. Heptafluorobutyric acid (HFBA) was used as a basic ion pairing agent for increased retention. An Atlantis C18 (150 × 4.6 mm, 3.5 μm) column was eluted with a mobile phase consisting of 0.1% heptaflurobutyric acid in water-acetonitrile (90:10, v/v). The analyses were performed at 40°C with a flow rate of 1 mL/min and CAD detection with Nitrogen operating gas pressure maintained at 35 psi. The method was validated for precision, linearity, and specificity. The procedure described here is simple, selective, and is suitable for routine quality control analysis.

Keywords: 4-Methanesulfonyl-piperidine (MSP), Charged aerosol detection (CAD), Heptafluorobutyric acid (HFBA), Method development, Method validation, Reversed phase HPLC

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INTRODUCTION

The determination of polar compounds is needed in many fields and often arises during pharmaceutical development. Polar analytes may include raw materials and intermediates used to synthesize drug substances or the drug substance itself. Most of the drug substance synthesis also involves the use of small molecule organic acids or bases lacking an UV chromophore as the starting material. The non chromophoric entities require the use of detectors like ELSD and CAD. Also, some of these polar molecules make reversed phase HPLC retention of them very difficult on typical reversed phase columns. In early stage development of drug substances, assessment of the purity of such compounds to be used in synthesis is of prime importance.

For example, BI had one drug in early stage development using 4-methanesulfonyl-piperidine hydrochloride salt as one of the starting materials. Retention of such polar compounds usually requires ion pairing reagents and highly aqueous mobile phase. From the quality control perspective in the pharmaceutical industry, it is important to develop and validate an analytical method for quantitative analysis of this starting material for release. The structure of this 4-methanesulfonyl-piperidine hydrochloride salt is shown in Figure 1.

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Over the past 20 years, several publications have shown that perfluorinated carboxylic acids are effective ion pairing reagents for the analysis of peptides, with the retention of a peptide increasing with chain length and concentration of the perfluorocarboxylic acid.[1–3] Although the use of TFA and other fluorinated acids as ion pairing reagents in the separation of peptides and proteins is generally recognized, HFBA as an ion pairing reagent in the separation of small ionizable molecules is not usually acknowledged and documented.[3,4] In many cases, it is not realized and used as a separation variable to control retention and selectivity for small molecule separation by RPLC.

Several techniques that include low wavelength UV, refractive index (RI), evaporative light scattering detection (ELSD), and chemiluminescent

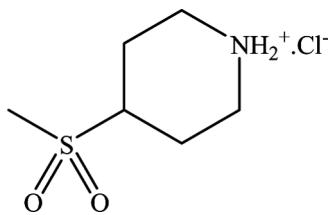

Figure 1. Structure of 4-Methanesulfonyl-piperidine hydrochloride salt.

nitrogen detection (based upon measuring bulk properties among diverse analytes) are employed for universal detection. Various characteristics of commercially available instruments employed for universal detection have been compared previously.[5–7] Low wave length UV provides higher sensitivity and improved gradient compatibility. Although UV detection remains the primary technique for many HPLC analyses, it is unable to detect compounds that lack a sufficient UV chromophore, such as many underivatized amino acids, lipids, carbohydrates, surfactants, polymers, and magnitude of response depending upon molar absorptivity, which can vary by orders of magnitude even among analogous structures. ELSD can detect compounds lacking a chromophore provided they have low volatility and has become widely used alone or to complement absorbance and mass spectrometry (MS) detectors.[7] Also, ELSD response magnitude often is less dependent on analyte chemical properties than MS or UV. However, it has significant limitations in precision, sensitivity, dynamic range, and the nature of calibration curves.[5,8,9] The demand for improved methods continues to drive novel approaches toward universal HPLC detection, such as those involving condensation nucleation light scattering[5] and inductively coupled plasma MS.[10]

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A new HPLC detection method has been developed based upon charged aerosol detection (CAD). This technique is based upon the coupling of HPLC with widely used electrical aerosol analyzer technology and is fundamentally different from that of other detectors.[8,11–14] The detection principle involves the charging of aerosol particles via corona discharge with subsequent electrometer based measurement and, thus, has some commonality with atmospheric pressure chemical ionization (APCI) MS. However, CAD operates by detecting charged particles that have a selected range of mobility rather than by measuring individual gas phase ions that are differentiated based upon m/z. Across a wide range, and does not depend significantly upon individual analyte properties.[13] This technique was successfully used for measurement of a variety of natural products,[15] carbohydrates,[16] peptides, lipids,[17] and also used for impurity analysis, excipient and formulation characterization, and in pharmaceutical cleaning validation.[18]

Herein, we describe the development and validation of an HPLC method with CAD detection for the quantitative determination of the above starting material. It was also important that the methodology could easily be adapted to other active pharmaceutical ingredients (APIs), starting materials, and isolated intermediates with no UV chromophore, thereby avoiding tedious sample (as salts) workup sometimes associated with the GC technique.

EXPERIMENTAL

Chemicals and Reagents

Acetonitrile (ACN) and Methanol (MeOH) was purchased from EMD (Gibbstown, NJ, USA). Water was purified using a Milli-Q purification system (Bedford, MA, USA). Heptafluorobutyric acid (HFBA), 99.9% reagent, was purchased from Aldrich Chemical Company Inc. (Allentown, PA, USA). 4-Methanesulfonyl-piperidine hydrochloride salt (starting material) and preceding intermediates were obtained from Nippon Chemical Industrial Co. (outsourced for Boehringer Ingelheim Pharmaceuticals Inc., Ridgefield, CT, USA).

Samples

4-Methanesulfonyl-piperidine hydrochloride salt and other preceding intermediates were manufactured at Nippon Chemical Industrial Co., Ltd according to their synthesis scheme. The standard sample of 4-methanesulfonyl-piperidine hydrochloride salt obtained was qualified as a reference substance with a purity of 99.6%.

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Chromatographic Conditions

The analyses were performed using high performance liquid chromatographic system HP 1100 system (Agilent Technologies, USA) driven by ChemStation 8.03® software. This system was composed of a quaternary pump (G1311A), an autosampler (G1329A), a mobile phase degasser (G1322A), and a diode array detector (G1315A) in line with corona charged aerosol detector (ESA Inc., Chelmsford, Massachussetts).

A reverse phase Atlantis dC_{18} (150×4.6 mm, 3.5 μm) column (Waters, Ireland) was used. All analyses were performed at the column temperature of 40°C under isocratic conditions with a mobile phase of 0.1% heptaflurobutyric acid in water/acetonitrile (90:10, v/v), an injection volume of 10 μL, flow rate of 1.0 mL/min and run time of 8 minutes. The nitrogen gas pressure was set at 35 psi and charged aerosol detector range to 100 pA for the CAD detector.

Preparation of Test Solution and Standard Solution

The test solution (0.4 mg/mL) was prepared by dissolving 20 mg of the MSP hydrochloride salt sample in 45 mL water:methanol (80:20, v/v) as diluent and diluting to 50 mL with the same. The standard solution

was obtained with the MSP hydrochloride salt reference substance prepared the same as the test solution, and further diluting to the required concentration with the mobile phase.

RESULTS AND DISCUSSION

Method Development

The initial experiment was performed on an Agilent 6890 Series GC equipped with an Agilent 7683B ALS (automated liquid sampler). Flame ionization detection (FID) was used. This compound is an HCl salt, and as such needs to be converted back to a free base in order to chromatograph on the GC. A method received from Chemical Development Group that used a 10/90 mixture of triethyl amine and tetrahydrofuran to convert the salt to the base was used. The column was an Rtx-5 Amine (30 m × 0.32 mm, 1 μm film thickness) from RESTEK (Bellefonte, PA, Cat. No. 12354). The oven temperature was increased at 25°C/min from 100°C to 280°C in 7.2 minutes and then held at 280°C for 4.0 minutes. The total run time was 11.2 minutes. The injector temperature was 200°C. Injection was carried out in the split mode at a split ratio of 30:1. The injection volume was 1 μL. Helium was used as the carrier gas at a constant flow rate of 1.8 mL/min. The FID temperature was 250°C.

Using this technique, we attempted to validate an assay method for the material. The standard preparation involves dissolving in 10% triethylamine (TEA) in THF and filtering with 0.5 μm PTFE membrane filter. It was observed that the method was not robust based on duplicate standard preparations. The typical % RSD of triplicate injections of a single standard were 1% or less. However, the % RSD obtained for 3 injections of two standard preparations (n = 6) was typically greater than 10%, indicating poor reproducibility in the standard preparations. Acceptance criteria for an assay method of this type are usually no more than 2% RSD for two standard preparations.

Using HPLC with charged aerosol detection, all the above sample preparation steps associated with GC were eliminated. Diluent used was 80/20 mixture of water and methanol. For increased retention of this ionic and polar analyte on a silica based line of difunctionally bonded reversed phase Atlantis dC18 columns (12% carbon loading), heptaflurobutyric acid (HFBA) was used as an ion pairing agent. HFBA was shown to be a useful volatile ion pairing reagent, which gives significantly increased retention of amine containing compounds over its shorter chain homologue trifluoroacetic acid. The effect of the concentration of the ion pairing reagent on the retention time was further investigated using HFBA at concentrations of 0.05, 0.1, 0.15, 0.20, and 0.25% in the mobile

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phase. The chromatographic conditions were kept the same as mentioned above in Instrumentation and Operating conditions section.

Before being fully implemented in the quantitative determination of MSP, this method was thoroughly validated for its specificity, linearity, precision, and robustness (based on analyte stability in standard and sample).

Discussion

As a quaternary ammonium salt, MSP exhibited little or no retention using the typical reversed phase separation. After adding 0.1% HFBA to the mobile phase, the retention time of MSP on the Atlantis dc18 column was altered to 4.40 min. Because, ion pair chromatography involves reversed phase separation and ion exchange mechanisms the uptake of the ion pairing reagents on the column mainly determines the retention of the analytes. At a given concentration, longer chain ion pairing results in longer retention. When the concentration of HFBA was increased to 0.25%, the retention time of MSP was 4.40 min, which is similar to the effect of HFBA at the lower concentration (0.25%). This indicated that the retention time of MSP could not be further altered due to the saturated uptake of the ion-pairing reagent on the column.

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Specificity

The specificity was evaluated by individual injection of the diluent blank, the working standard solution, and preceding intermediates isolated during synthesis of the analyte. No interference was observed at the same or at ±5% of retention time of the analyte, and preceding intermediates did not elute under isocratic conditions of the method.

Linearity

The linearity plot was generated from five concentration levels of 320, 360, 400, 440, and 480 μg/mL and the corresponding peak areas. Solutions corresponding to each concentration level were injected in duplicate. It demonstrated a good linearity in a range of 320–480 μg/mL for MSP. The linear equation for the calibration curve was $y = 5205.6x + 785100$ with a correlation coefficient (r) of 0.9937.

Precision

Method reproducibility was determined by measuring repeatability and precision. Repeatability of the method was determined by calculating

Table 1. Repeatability study data for 4-methanesulfonyl-piperidine

Injection no.	RT (min)	4-Methanesulfonyl-piperidine calibration factor (μV.s/mg)
1	4.47	137559
2	4.46	142437
3	4.46	143422
4	4.47	143690
5	4.47	145402
6	4.47	143117
Average (n = 6)	4.47	142605
% RSD	0.04	1.9

% RSD (n = 6, 3 injections for each preparation) for the average calibration factor from duplicate preparations of MSP standard solution at 100% test concentration (400 μg/mL). The % RSD values of the results corresponding to the retention times and calibration factors were found to be 0.04% and 1.9%, respectively (listed in Table 1).

Six preparations from the same lot were analyzed for reproducibility. Single injection was made for each preparation. The assay precision, from the results shown was less than 0.50% RSD (listed in Table 2).

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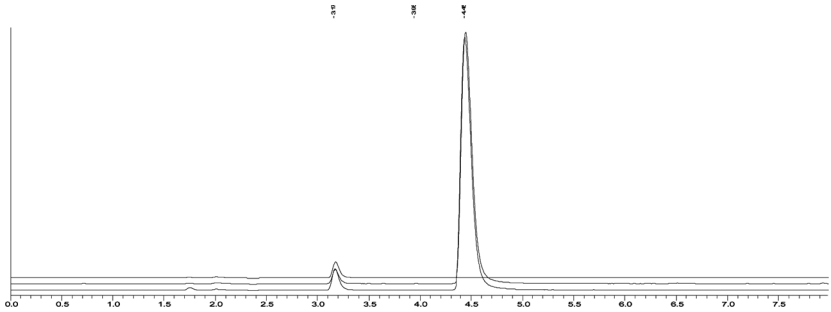
Robustness (Stability of Analyte Solution)

The solution stability of the standard/sample (0.4 mg/mL) preparation was monitored by comparing the average calibration factor of the peak of interest for standard and assay values for sample. The RSD (%) for the average calibration factor of all standard solutions, injections, and

Table 2. Sample precision (reproducibility) data for 4-methanesulfonyl-piperidine

Preparation	4-Methanesulfonyl-piperidine assay (%, w/w)
1	101.4
2	100.7
3	101.0
4	101.4
5	100.2
6	100.6
Average (n = 6)	100.8
% RSD	0.4



Substance	RT*
Diluent peak	3.2
4-Methanesulfonyl-piperidine	4.5

RT = approximate retention time in minutes

Figure 2. Overlay of diluent, standard and sample chromatogram (full scale).

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assay (%) values of all sample solution injections over a period of 23 hours were within 3% RSD.

Sample Analysis

For the purpose of verification, two batches were analyzed according to this procedure. Two sample preparations for each lot and one injection per preparation were made. Overlay of the representative blank, standard, and sample chromatograms are shown (Figure 2). The average results for each batch are listed in Table 3.

CONCLUSION

The method has been demonstrated to be selective, precise, and robust (analyte stability). No matrix related interference was encountered. Since the methodology has none of the sample preparation restrictions

Table 3. Sample analysis

Sample ID	Assay (%)
Lot 1	100.8
Lot 2	100.6

encountered in the GC technique, especially for salts (neutralization followed by extraction), HPLC/CAD detection is used as an alternative approach to measure virtually any non-volatile analyte (small molecule starting materials and synthetic intermediates) and APIs with no UV chromophore.

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REFERENCES

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1. Daas, C.; Mahalakshmi, P.; Grandberry, D. Manipulation of ion pairing reagents for reversed phase high performance liquid chromatographic separation of phosphorylated opioid peptides from their non-phosphorylated analogues. J. Chromatogr. A **1994**, *678*, 249–257.
2. Starratt, A.N.; Stevens, M.E. Ion-pair high-performance liquid chromatography of the insect neuropeptide proctolin and some analogs. J. Chromatogr. **1980**, *194*, 421–423.
3. Guo, D.; Mant, C.T.; Hodges, R.S. Effects of ion-pairing reagents on the prediction of peptide retention in reversed-phase high-resolution liquid chromatography. J. Chromatogr. **1987**, *386*, 205–222.
4. Sasagawa, T.; Okuyama, T.; Teller, D.C. Prediction of peptide retention times in reversed-phases high-performance liquid chromatography during linear gradient elution. J. Chromatogr. **1982**, *240*, 329–340.
5. Allgeier, M.C.; Nussbaum, M.A.; Risley, D.S. Comparison of an Evaporative Light-Scattering Detector and a Chemiluminescent Nitrogen Detector for Analyzing Compounds Lacking a Sufficient UV Chromophore. LCGC. **2003**, *21* (4), 376–381.
6. Petritis, K.; Elfakir, C.; Dreux, M. A comparative study of commercial liquid chromatographic detectors for the analysis of underivatized amino acids. J. Chromatogr. A. **2002**, *961* (1), 9–21.
7. Yurek, D.A.; Branch, D.L.; Kuo, M.S. Development of a system to evaluate compound identity, purity, and concentration in a single experiment and its application in quality assessment of combinatorial libraries and screening hits. J. Comb. Chem. **2002**, *4* (2), 138–148.
8. Dixon, R.W.; Peterson, D.S. Development and testing of a detection method for liquid chromatography based on aerosol charging. Anal. Chem. **2002**, *74* (13), 2930–2937.
9. Korpochok, J.A.; Magnusson, L.E.; Heybroek, M.; Sadain, S.; Yang, X.H.; Anisimov, M.P. Fundamental aspects of aerosol-based light-scattering detectors for separations. Adv. Chromatogr. **2000**, *40*, 275–314.

10. Lawless, P.A. Particle charging bounds, symmetry relations, and an analytic charging rate model for the continuum regime. J. Aerosol Sci. **1996**, *27*, 191–215.
11. Medved, A.; Dorman, F.; Kaufman, S.L. A new corona-based charger for aerosol particles. J. Aerosol Sci. **2000**, *31* (SUPP/1), 616–S617.
12. Liu, B.Y.H.; Pui, D.Y.H. On the Performance of the Electrical Aerosol Analyzer. J. Aerosol Sci. **1975**, *6*, 249–264.
13. Flagan, R.C. History of Electrical Aerosol Measurements. Aerosol Sci. Technol. **1998**, 28.
14. Alpert, A.J. Hydrophilic-interaction chromatography for the separation of peptides, nucleic acids and other polar compounds. J. Chromatogr. **1990**, *499*, 177–196.
15. Fukushima, K.; Kanedai, Y.; Hirose, K.; Matsumoto, T.; Hashiguchi, K.; Senda, M.; McCarthy, R.; Asa, D.; Gamache, P.; Acworth, I. HPLC with Charged Aerosl detection for the measurement of Natural Products. Chromatography **2006**, *27* (1), 83–86.
16. Asa, D. Carbohydrate and oligosaccharide analysis with a universal HPLC detector. American Laboratory **2006**, *38* (7), 16–18.
17. Moreau, R.A. The Analysis of Lipids via HPLC with a Charged Aerosol Detector. Lipids **2006**, *41* (7), 727–734.
18. Forsatz, B.; Snow, N.H. HPLC with charged aerosol detection for pharmaceutical cleaning validation. LCGC **2007**, *25*, 960–968.

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