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Use of ion chromatography for the verification of drug authenticity

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Abstract

Investigations of drug authenticity focus on both bulk drugs and finished products. Excipients and contaminants from manufacturing processes may be used as "chemical fingerprints" to track drug sources. This paper describes the ion chromatographic determination of sodium lauryl sulfate, chloride, phosphate and citrate in drug formulations as applied to drug authenticity cases.

1. Introduction

In recent years, the quality and authenticity of drugs, especially generics, have come under intense public scrutiny. The Food and Drug Administration (FDA) has legal and scientific processes, such as the New Drug Application (NDA) and Abbreviated New Drug Application (ANDA), by which it approves new and generic drugs. However, the agency must ensure that drugs are produced only by approved manufacturers, and that the formulations and processes which have been approved are followed.

Methodology to detect contaminants in drugs is necessary for investigations of authenticity. Analysis of contaminants in bulk drugs may be used as a "chemical fingerprint" to track bulk drugs since various manufacturing processes may contribute characteristic residual chemicals to the fingerprint. Several investigators have used analysis of contaminants to distinguish between samples: Neumann and Gloger [1] utilized capillary gas chromatography for the analysis of impurities in heroin, and Wolnik *et al.* [2] used inductively coupled plasma–optical emission spectroscopy to distinguish between manufacturers of cyanide in Tylenol tamperings.

Investigations of authenticity may also focus on finished products. Manufacturing processes use distinct excipients: buffers such as phosphate and citrate in injectables, fillers such as mannitol, sorbitol, dibasic calcium phosphate, calcium sulfate and lactose, and lubricating agents such as sodium lauryl sulfate in tablets [3]. The absence or presence of various excipients may indicate deviations from approved formulations and/or processes, or counterfeit products.

Ion chromatography is an important analytical technique in forensic investigations of drug authenticity. In this paper, two cases in which ion chromatography was used to discrimin-

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ate between investigative samples will be discussed.

2. Experimental

2.1. Apparatus

The instrumentation used included a Dionex (Dionex, Sunnyvale, CA, USA) 4500 ion chromatograph with gradient pump, Rheodyne Model 9126 injector (10-μl loop), pulsed electrochemical detector in the conductivity mode, automated sampler module, and AI-450 software program for data collection and calculation. The column used was a Dionex Omnipac PAX-500, 250×4 mm and the suppressor was an Anion Micromembrane Suppressor (AMMS) II, also from Dionex.

2.2. Reagents, standards and samples

Water used in these studies was purified using a Millipore (Bedford, MA, USA) Milli-Q system. Eluents were prepared from 50% (w/w) aqueous sodium hydroxide, Optima-grade methanol and acetonitrile (Fisher Scientific, Fair Lawn, NJ, USA). Standards were prepared with sodium lauryl sulfate and citric acid monohydrate (Sigma, St. Louis, MO, USA) and certified ion chromatography anion standard mixture (Dionex).

Sodium lauryl sulfate (SLS) stock standard was prepared from SLS dissolved in methanol. Working standards were prepared by appropriate dilutions of the stock with methanol. All other standards were prepared in distilled deionized water (DDW).

Tablet coatings were physically removed by scraping or peeling. The remainder of each tablet was reduced to powder and three tablets composited together. Three portions of each composite were accurately weighed and extracted with methanol, then filtered through 0.2-μm nylon 66 syringe filters. All other samples were diluted with DDW and filtered through 0.2-μm nylon 66 syringe filters.

3. Results and discussion

3.1. Sodium lauryl sulfate in generic human drug case

Before new drug formulations are marketed, extensive testing is required to prove both efficacy and safety. Generics, however, require less testing since the innovator has already characterized the active drug. Manufacturers must document manufacturing processes, and manufacturers of generics must also submit finished product from pilot batches for bioequivalence testing. Any formulation differences which exist between the innovator and generic product must not affect the bioavailability and safety of the drug.

Excipients include all of the ingredients in a finished product other than the active ingredient. Many types of excipients are used including binders, fillers, disintegrating agents, lubricants, flavors and sweetening agents [3]. Excipients can affect the disintegration rates of tablets and the bioavailability of water-soluble drugs. As such, changes in the manufacturing process are not allowed after a drug has been approved unless an amendment to the manufacturer's application is approved.

Suspicions arose from investigative information that a manufacturer's lot submitted for bioequivalence testing (the biolot) was not produced by the same formulation as the marketed product. Tablets can be prepared by three different methods: wet granulation in which ingredients are mixed in a slurry, dry granulation which involves the compaction of powders at high pressure, and direct compression [3]. Each of the methods has both advantages and disadvantages. It was suspected that SLS, a lubricant, was used in the formulation of the marketed product, but not in the biolot formulation. Tablets were analyzed for SLS to determine if the biolot and production lots were the same.

An existing method for the analysis of linear alkyl sulfates in surfactants by ion chromatography was used to quantitatively determine SLS [4]. Gradient conditions are listed with Fig. 1. The tablets in question were also analyzed concurrently at FDA Division of Drug Analysis

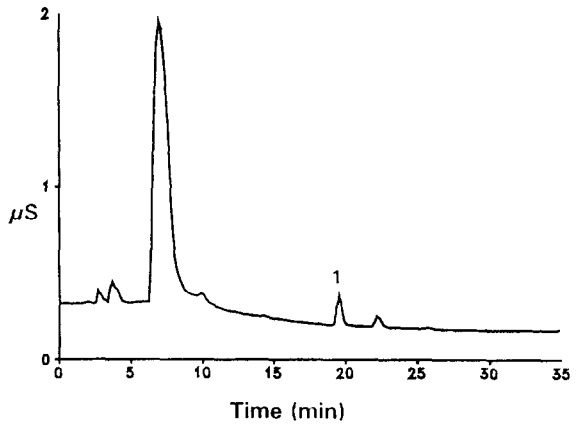

Fig. 1. Determination of sodium lauryl sulfate in a tablet. Peak 1 = SLS. Column: Omnipac PAX-500; flow-rate: 1.0 ml/min. Gradient program: eluent 1: 18 MΩ water; eluent 2: acetonitrile–water (90:10); eluent 3: 200 m*M* NaOH; eluent 4: methanol–water (45:55). Suppressor: AMMS-II; regenerant: 12.5 m*M* sulfuric acid at 10 ml/min.

Time (min)	Eluent 1 (%)	Eluent 2 (%)	Eluent 3 (%)	Eluent 4 (%)
0.0	80	0	10	10
20.0	30	50	10	10
40.0	10	70	10	10
50.0	10	70	10	10
50.1	80	0	10	10
60.0	80	0	10	10

using a modification of a US Pharmacopeia method [5]. SLS was converted to lauryl alcohol and determined in that form by GC–MS. SLS was positively identified by GC–MS in all samples except for the biolot, but was not quantitated.

Calibration curves of SLS prepared in methanol were linear in the range studied (20–200 μg/ml) with a slope of 27 527 area response counts per μg/ml, *y*-intercept −189 428 area response counts, and correlation coefficient equal to 0.9995. Repeatability of response and retention time were 3.1 and 0.8% relative standard deviation, respectively. A limit of detection (LOD) was not calculated statistically due to the slope of the baseline but was defined as the lowest concentration of standard for which a definitive peak was observed. Solution LOD was 2 μg/ml, equivalent to a LOD in the tablets studied of 40 μg SLS per gram tablet.

The SLS determined in several lots of the tablet in question from the same manufacturer are presented in Table 1. Three samples were weighed and extracted from each lot (four from lot C). The amount of active drug in the tablets (5 mg *versus* 10 mg) did not affect the analysis of SLS. Spike recoveries were performed to determine the effectiveness of the methanol extraction. Although SLS is soluble in water [6], no SLS was detected in the aqueous extracts of the tablets. The SLS concentration was 0.3 m*M* in the aqueous extracts, which is below the critical concentration for micellar formation. Perhaps the interaction of SLS with the other ingredients in the tablet prevented extraction with water. The percent recovery from methanol was very dependent upon the method of spike preparation. The first method consisted of adding 2.2 mg SLS to 0.4 g of composited tablet. A 0.05-g portion of the spiked sample was extracted with 2 ml of methanol. The sample was then filtered and injected onto the Omnipac column. Recovery of SLS through this procedure was only an average of 60%. The second method, however, had an average spike recovery of 92%. In this method, 1.1 mg SLS was added to a smaller portion of composited tablet (0.2 g), but the entire spiked sample was extracted with 5 ml of

Table 1
Concentration of sodium lauryl sulfate in drug tablets

Lot[a]	10 mg active μg SLS/g tablet	Lot	5 mg active μg SLS/g tablet
A	2396	D	2960
	2229		3092
	2352		2923
B	3093		
	3374		
	2864		
C	2984		
	3008		
	2831		
	2911		

[a] Three to four samples prepared per lot.

methanol. This extract was then diluted 1:1 with methanol and filtered through a nylon 66 filter. It is suspected that the poor recovery of the first spike method was due to incomplete mixing of the SLS with the sample. Since the entire sample was extracted in the second method, there were no homogeneity problems. The distribution of SLS in the finished tablets from the manufacturer was homogeneous since the percent relative standard deviation between multiple samplings of each lot range from 2.7 to 8.2% R.S.D.

No SLS was detected, either by ion chromatography or GC–MS, in the lot submitted for bioequivalency testing. However, SLS was determined by ion chromatography at an average level of 2584 μg/g tablet in the finished product. The limit of detection for SLS in the tablet was 65 times greater than the level of SLS declared. It was concluded from this information, in conjunction with other chemical analyses and investigative information, that the biolot was not produced by the same formulation as the marketed product. Although the presence or absence of SLS, an approved excipient in drugs, may not seem to be a major issue, it is very important that manufacturers follow the master formulations which have been approved by FDA and tested for bioequivalency.

3.2. Anions in veterinary drug case

It was evident from the very first analysis in the following case, that fraud was involved. The sample, a liquid veterinary drug, did not contain the active drug which was declared on the label. However, the details of the operation were not as evident and ion chromatography proved to be a valuable technique in piecing together the details.

The suspect samples contained a different active ingredient which is used to treat the same medical condition as the falsely declared drug. The substitute drug, however, is less expensive, and not as potent as the labeled drug. The motive for fraud became clear. Package the cheaper drug as the more expensive labeled drug and a greater profit will be made. If the counterfeiter had not put any active ingredient in his product, the scam would have quickly failed. In order to make a stronger legal case, it was important to find the source of material in the counterfeit containers.

The substitute drug is available as either the hydrochloride or the phosphate salt. Using this information, three sets of samples were analyzed on an Omnipac Pax-500 column. The solvent resistance of this column was necessary in order to minimize sample preparation and possible damage the organic drug might do to an ion chromatography column. Samples were diluted sufficiently that filtration was the only sample pretreatment necessary. The column was cleaned with 90% aqueous acetonitrile. The three sets of samples consisted of: legitimate substitute drug from a manufacturer who had sold product to the suspect distributors, samples confiscated from warehouses, and suspect samples confiscated from dealers.

Fig. 2 illustrates the separation of chloride, sulfate, phosphate and citrate in a suspect sample. Calibration curves were linear (correlation coefficients >0.9990) in the ranges studied (Cl^- 0.7–30 μg/ml; PO_4^{3-} 3–155 μg/ml; citrate 5–50 μg/ml). Plots were constructed comparing chloride concentration *versus* the concentration (expressed in molarity) of substitute drug (refer to Fig. 3); phosphate concentration *versus* the con-

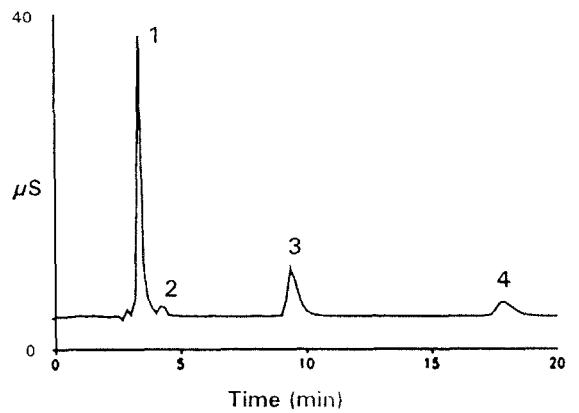

Fig. 2. Separation of anions in a veterinary drug on the Omnipac PAX-500 column. Eluent: 40 m*M* NaOH–5% methanol; flow-rate: 1.0 ml/min; suppressor: AMMS-II; regenerant: 12.5 m*M* sulfuric acid at 5 ml/min. Peaks: 1 = chloride; 2 = sulfate; 3 = phosphate; 4 = citrate.

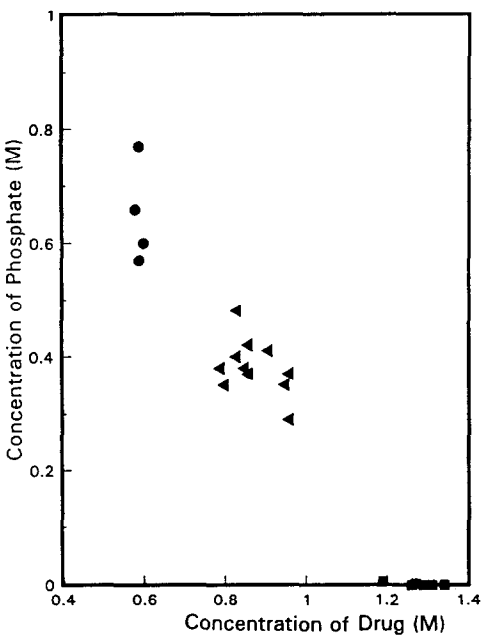

Fig. 3. Veterinary drug: molarity of phosphate plotted against molarity of substitute drug for three sets of samples. ● = Legitimate; ◀ = suspect; ■ = warehouse.

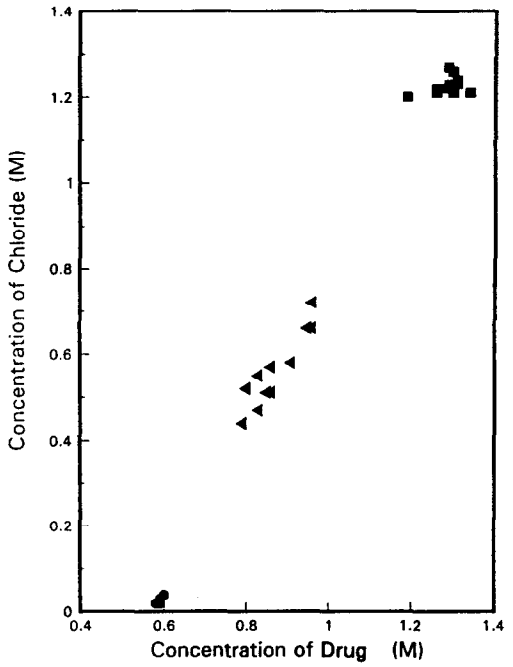

Fig. 4. Veterinary drug: molarity of chloride plotted against molarity of substitute drug for three sets of samples. Symbols as in Fig. 3.

centration of substitute drug (refer to Fig. 4); and citrate concentration *versus* the concentration of substitute drug (refer to Fig. 5). Citrate is a commonly used buffer in certain veterinary drugs. In each of the plots a trend was noted: the data divided into three distinct groups well separated from each other. The legitimate product had equimolar phosphate and active (of substitute drug) concentration, no chloride and the highest level of citrate. Samples seized from warehouses, however, all had low phosphate and citrate, but had equimolar substitute drug and chloride levels. Suspect samples packaged as the more expensive drug all had levels of phosphate, citrate, chloride and substitute drug which were in between the legitimate substitute drug and warehouse samples. The suspect samples were mislabeled, since they contained none of that active ingredient, however, they also contained counterfeit substitute product.

Legitimate product contained approximately 0.6 *M* substitute drug in the phosphate form and 0.2 *M* citrate, while the warehouse samples

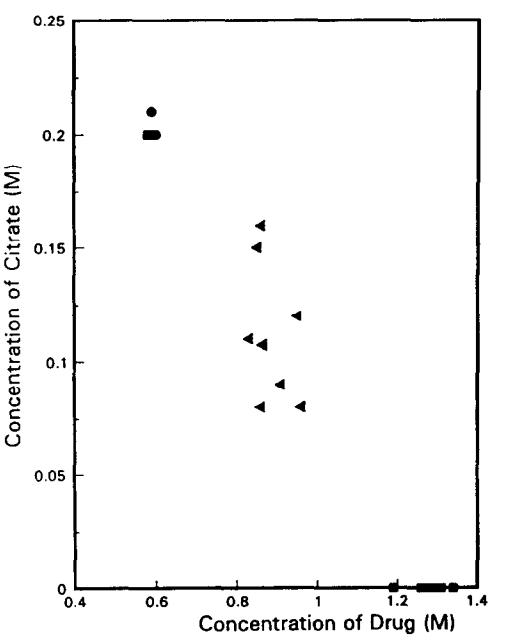

Fig. 5. Veterinary drug: molarity of citrate plotted against molarity of substitute drug for three sets of samples. Symbols as in Fig. 3.

Table 2
Comparison of chloride, phosphate, citrate and substitute drug concentrations in veterinary drug samples

Sample	Cl^- (*M*)	PO_4^{3-} (*M*)	Substitute drug (*M*)	Citrate (*M*)
Legitimate[a]	ND[b]	0.6	0.6	0.2
Warehouse[c]	1.2	ND	1.2	ND
Legitimate/2[d]	ND	0.3	0.3	0.1
Warehouse/2[d]	0.6	ND	0.6	ND
Legitimate + warehouse 1:1	0.6	0.3	0.9	0.1
Suspects[e]	0.6	0.4	0.9	0.1

[a] Average of four samples
[b] ND = Not detected.
[c] Average of fifteen samples.
[d] For purposes of explanation, concentrations of legitimate or warehouse were divided by two.
[e] Average of eleven samples.

contained the drug in the hydrochloride form (1.2 *M*) only. On average, the suspects contained 0.9 *M* substitute drug, 0.4 *M* phosphate, 0.6 *M* chloride and 0.1 *M* citrate. The suspect samples had the same concentrations as if the legitimate material and warehouse samples had been mixed one to one (refer to Table 2). Based upon the described plots, as well as packaging, investigative information, and results of other chemical analyses, the following conclusion was made. Suspects were adding warehouse material to legitimate substitute drug to increase its potency, then packaging it as the more expensive drug.

4. Conclusions

Most drug manufacturers adhere to the law and follow good manufacturing practices. However, counterfeit and unscrupulous manufacturers do exist. In order to ensure that drugs are produced only by approved manufacturers and with approved formulations, our analyses must become more and more sophisticated. The utility of ion chromatography was demonstrated in two cases by the analysis of sodium lauryl sulfate, chloride, phosphate and citrate.

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