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The Analytical Approach

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Over-the-Counter Drug Analyses with HPLC

The complexity of over-the-counter (OTC) drug preparations makes the identification of their active constituents extremely difficult. Unlike the single compound ethical drug often found in prescription preparations, OTC products are frequently formulations complicated by the numbers and relative quantities of active ingredients as well as by a large variety of excipients. Excipients include antioxidants, emulsifiers, stabilizers, flavoring agents, and buffers.

The most effective analytical approach followed in this laboratory for analyzing OTC products complements high performance liquid chromatography (HPLC) with various spectrometries, specifically the mass spectrometries (electron impact, chemical ionization, field desorption, GC/MS) and IR, UV, and NMR (^{13}C , ^1H) spectrometries. Application of these analytical techniques permits the conclusive identifications of compounds required for compliance with safety and efficacy regulations.

The use of HPLC obviates earlier complex, time-consuming, and less specific approaches, viz., liquid-liquid extractions and colorimetric assays. The knowledge obtained in analyzing OTC preparations also serves as a basis for solving other pharmaceutical problems.

Examples of these HPLC approaches to the analyses of OTC preparations are given below. Initially, HPLC was applied to the analysis of ethical products such as antibiotics. HPLC columns used for these assays were packed with medium-efficiency 37- μm pellicular materials. The introduction of high-efficiency 5- and 10- μm packings gave the increased resolution and greater sensitivity required for stability-indicating methods. The development of ion-pairing techniques for reversed phase systems permitted the analysis of many pharmaceutical compounds that were previously difficult

to analyze with an ion-suppression reversed phase system because of the pH restrictions (pH 2–8) on the columns. The current method permits the separation of active compounds from their decomposition products, precursors, contaminants, and excipients. One major advantage of the technique is the simplicity of sample preparation, which is due to the high resolving power of HPLC for complex mixtures. Extensive pretreatment is seldom needed, most samples requiring only dissolution, dilution, and injection. The methods are precise ($RSD_{1\sigma} < 1.5\%$) and accurate.

OTC Choline Salicylate Product

One OTC product that was analyzed contained choline salicylate. Choline salicylate is an aspirin substitute and is stable in aqueous solution. The HPLC method developed for the salicylate used camphorsulfonic acid in the mobile phase to completely suppress salicylate ionization.

Since both the starting materials and formulations are viscous liquids, we wanted to establish a procedure for measuring samples volumetrically. This was done by filling a "to contain" pipet to the mark with sample, allowing it to drain into a volumetric flask, and then carefully rinsing the material adhering to the wall of the pipet into the flask with mobile phase liquid. A known amount of internal standard was added, and the sample diluted to volume. To prove that this technique was valid, the precision of the volumetric measurement was compared to that obtained with weighed samples. The average values obtained for both techniques were identical with RSDs of 0.8% and 0.7%, respectively. Thus, no problem appeared to occur with the volumetric measurement.

Attempts were made to decompose choline salicylate in controlled experiments. The sample was subjected to heat, UV radiation, and complexing

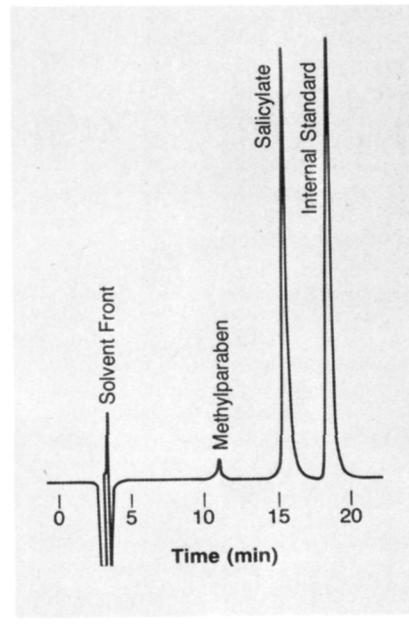


Figure 1. HPLC separation of salicylate in a choline salicylate formulation. Column, Dupont Zorbax C₈ (25-cm X 4.6-mm i.d.); AUFS, 0.04; flow rate, 1.0 mL/min; detector, 280 nm; injection, 10 μL ; mobile phase (50:50) methanol: 0.025 M camphorsulfonic acid

and oxidizing agents. The HPLC and TLC (thin layer chromatography) chromatograms showed no decomposition products. Thus, salicylate appeared to be exceptionally stable under these conditions.

A chromatogram of the separation is shown in Figure 1. Methyl paraben is a preservative in the formulation. Ethyl paraben is the internal standard. The purity of the salicylate peak was investigated by an absorbance ratio technique (1). Absorbance ratios are determined by measuring the absorbance of the peak at several wavelengths and calculating the ratios of the absorbances. These ratios are con-

stants for pure compounds. The ratios were determined on three samples—a reference standard; a 6-month, 50 °C sample of starting material; and a 6-month, 50 °C formulation. The absorbance ratios of the samples showed no significant variations. While this experiment did not completely eliminate the possible contribution of additional components to the peak, it was strongly indicative that a single entity was present, and we felt that the method had been validated.

Samples were analyzed for several months without any problems. Then, starting material samples that had been stored for 6 months were received. A sample stored at 50 °C assayed 1.9% higher in choline salicylate than did a sample stored at 5 °C. The assay on the 5 °C sample was in agreement with the result on the original sample. If decomposition had occurred at the higher temperature, it probably would have caused low, not high, results. Absorbance ratio measurements and TLC evidenced no decomposition products that might interfere. No inorganic material was present. IR and MS data showed no significant differences. Since the samples were in solution, the possibility of water loss was considered. Karl Fischer analyses for water solved the problem. A material balance was obtained on both samples—99.8% on the 5 °C sample and 99.2% on the 50 °C sample. The change in choline salicylate assay value was compensated for by the change in water content.

OTC Allantoin Product

Another OTC product analyzed was a skin healing and protecting lotion that contained allantoin. The complexity of the matrix precluded the use of frequently applicable colorimetric and titrimetric procedures, and the literature described no appropriate HPLC methods. We therefore developed an HPLC procedure specific for allantoin that included the use of a variable wavelength UV detector set at 220 nm.

The initial investigation included testing a variety of HPLC reversed phase columns, including Waters μBondapak C₁₈, Dupont Zorbax C₈ and C₁₈, and Whatman Partisil-10 ODS-2. Allantoin, however, was not retained on any reversed phase column—even those with very heavy carbon loading. Nor was ion pairing or ion exchange HPLC applicable because allantoin was not retained by any of these columns.

Since none of these approaches was successful, the next step was to investigate bonded normal phase systems. Bonded normal phase columns tolerate water well. The fact that allantoin is very water-soluble and only sparingly soluble in methanol made these columns appear promising for separation of compounds as highly polar as allantoin. Cyano columns, one of the most common types of normal phase columns, were tried first.

Column packings having cyano functionality only did not retain allantoin. One packing, however, Whatman PSX 10/25 PAC, has some amino functionality in addition to cyano, and it retained the compound slightly. We decided to try packings having amino groups.

Both Waters μBondapak and DuPont Zorbax amino columns gave satisfactory retention and peak shape. Figure 2 is a chromatogram of the separation of an allantoin formulation on a Waters column using a water-acetonitrile (11:89) mobile phase. Many components in the formulation also absorb at 220 nm, but these are well separated from allantoin.

The multiplicity of ingredients in the formulations caused other difficulties besides separation problems. Sample preparation is vital to the success of this type of analysis. Large differences in the solubility of components formulated with allantoin presented one of the most difficult problems encountered in the HPLC analysis of these lotions. To successfully assay these preparations, the sample must be completely solubilized or separated from insoluble material by filtration, centrifugation or other means to prevent column blockage by insoluble material at the head of the column. Allantoin lotions were particularly difficult to deal with.

It was observed that certain formulations did not dissolve in the mobile phase which was the preferred diluent. Attempts to solubilize the formulations by heating in methanol were unsuccessful because of decomposition detectable by the presence of extraneous peaks in the chromatogram. Tetrahydrofuran (THF) was a good solvent for the matrix, so mixtures of THF and water were tried. A 30:70 mixture of these solvents kept allantoin in solution and dissolved most of the remaining components. While the solution was slightly cloudy, any undissolved material settled on standing, and the supernatant liquid was suitable for injection. Samples of spiked placebo were assayed to validate the method. The recoveries averaged 99.6% with an RSD of 1.1%.

An additional problem arose when a component in one of the placebo mixtures was found to elute at exactly the same retention volume as allantoin. This interference would have caused allantoin results to be 10% too high. Attempts to modify the chromatographic conditions to solve this problem—first by changing the mobile

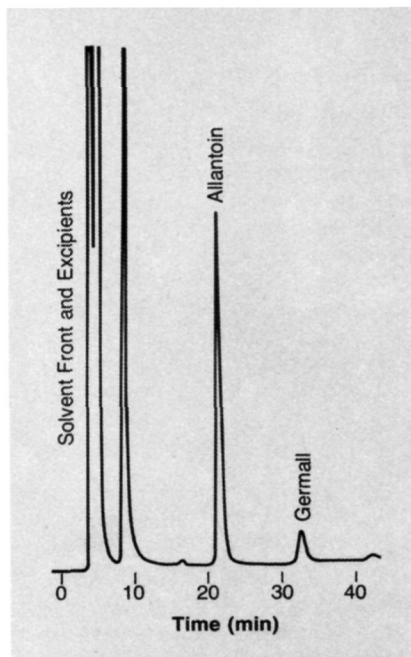


Figure 2. HPLC separation of allantoin in a lotion. Column, Waters μBondapak NH₂ (30-cm × 3.9-mm i.d.); AUFS, 0.08; flow rate, 1.0 mL/min; detector, 220 nm; injection, 25 μL; mobile phase, (11:89) water:acetonitrile

phase and then by employing a completely new system on a silica column—were not successful. Extractions from acidic, neutral, and basic solutions were also unsuccessful in removing the interference. An attempt was made to identify the source of the problem by investigating the constituents of the placebo. Germall 115, a preservative in the lotion, is a condensation product of allantoin and formaldehyde (2). To determine if this excipient was the source of the HPLC interference, a sample of Germall 115 was injected into the instrument. A small peak eluted at the retention volume of allantoin. To identify the chemical composition of the unknown peak, the placebo was injected, and the unknown peak was collected and examined by mass spectrometry.

Electron impact (EI) mass spectrometry indicated that the spectrum was essentially identical to that of a reference sample of allantoin. To verify further that the peak was allantoin, a field desorption (FD) mass spectrum was obtained. Since FD is a soft ionization technique, many compounds that are easily fragmented by EI retain their molecular integrity. Field desorption mass spectrometry showed a molecular ion peak at *m/e* 158, which corresponds to that expected for allantoin. This particular lot of Germall 115 appeared to contain some unreacted precursor.

Many formulations had been prepared with this lot of material, and assays were required. The amount of interference in the placebo was found to

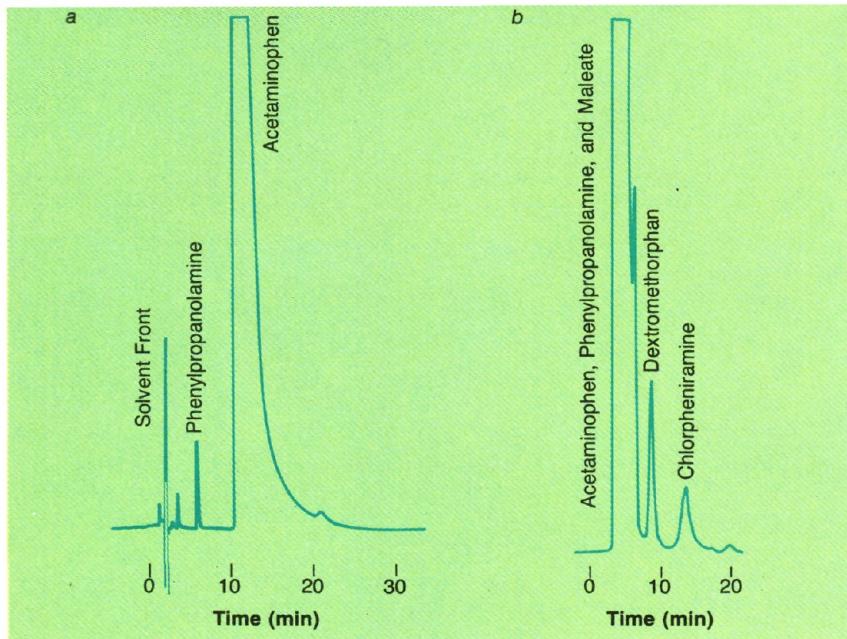


Figure 3. (a) HPLC separation of phenylpropanolamine hydrochloride and acetaminophen in a dissolution sample. Column, Whatman PXS-1025 ODS (25-cm \times 4.6-mm i.d.); AUFS, 0.02; flow rate, 1.2 mL/min; detector, 256 nm; injection, 20 μ L; mobile phase, (9:91) methanol:0.01 M methanesulfonic acid. (b) HPLC separation of dextromethorphan hydrobromide and chlorpheniramine maleate in a dissolution sample. Column, Waters μ Bondapak C₁₈ (30-cm \times 3.9-mm i.d.); AUFS, 0.02; flow rate, 1.0 mL/min; detector, 262 nm; injection, 100 μ L; mobile phase, (75:25) methanol:H₂O (0.02 M dioctylsulfosuccinate sodium salt, 0.1% phosphoric acid)

correspond to the amount of allantoin expected from the theoretical content of Germall 115. The formulations were corrected by subtracting the equivalent values for allantoin calculated from the Germall concentrations from the total allantoin content of the samples. The placebo was monitored with the stability samples so that any change in allantoin content would be observed and the proper correction made for the samples.

OTC Cough-Cold and Allergy Products

To illustrate the uses of HPLC in the analysis of multicomponent products, ion-pairing reversed phase procedures used for some of these complex formulations are described. One product was a cough-cold remedy that contained phenylpropanolamine hydrochloride, acetaminophen, chlorpheniramine maleate and dextromethorphan hydrobromide. It was necessary to obtain assay and dissolution data on original and stability samples of the solid dosage forms. The extremely dilute (one capsule or tablet/900 mL) solutions of dissolution media were difficult to analyze. To define the solubility characteristics of the preparations, the dissolution medium was sampled at varying time intervals. A large number of samples of low concentration were generated. The HPLC instruments were found to have sufficient

sensitivity and stability to assay these solutions successfully. The automated assay procedure permitted samples to be analyzed unattended overnight.

Because of the marked differences in polarity of the four compounds, two HPLC systems were used for the analysis of the cough-cold remedy. Figures 3a and 3b are chromatograms of a dissolution sample at $t = 15$ min. Depending upon the chain length of the ion-pairing reagent employed and the methanol content, it was possible to have phenylpropanolamine elute before or after acetaminophen. With a short chain reagent (such as methanesulfonic acid) and 9% methanol, phenylpropanolamine eluted before acetaminophen. This was desirable because of differences in concentration and UV response of the two compounds. Phenylpropanolamine and acetaminophen eluted at the solvent front when dioctylsulfosuccinate was used as the ion-pairing reagent in a mobile phase containing 80% methanol. In the same system, the less polar compounds, dextromethorphan and chlorpheniramine, were retained and quantitated.

Another multicomponent product, an allergy remedy, contained phenylpropanolamine hydrochloride, chlorpheniramine maleate, and aspirin. A single HPLC system with a camphorsulfonic acid mobile phase separated these compounds and salicylic acid, the decomposition product of aspirin.

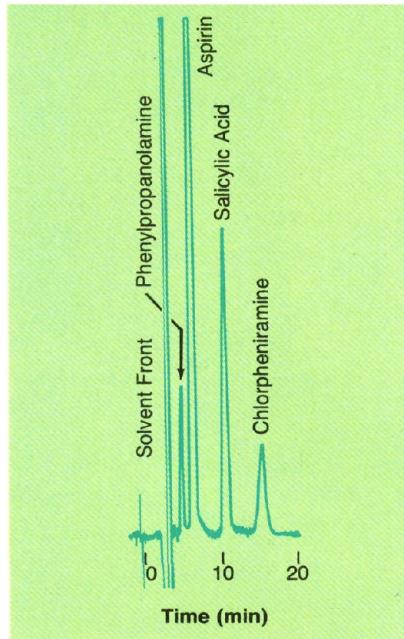


Figure 4. HPLC separation of phenylpropanolamine, aspirin, salicylic acid, and chlorpheniramine in an allergy remedy. Column, Waters μ Bondapak C₁₈ (30-cm \times 3.9-mm i.d.); AUFS, 0.04; flow rate, 1.0 mL/min; detector, 256 nm; sample, 20 μ L; mobile phase (40:60) methanol:H₂O (0.0125 M camphorsulfonic acid)

A chromatogram of the separation on a Waters μ Bondapak C₁₈ column is shown in Figure 4. Aspirin hydrolyzes rapidly to salicylic acid in an aqueous system and cannot be quantitated in overnight runs. However, it can be quantitated if each sample is prepared shortly before injection. A manual UV assay for aspirin and salicylic acid was found to be more convenient than the HPLC system and required less overall operator time.

To summarize, the reliability of HPLC methods supplemented by other analytical techniques for the analysis of OTC products has been investigated. The examples illustrated in this paper show the utility and advantages of this effort. Large numbers of samples can be assayed automatically and complex mixtures separated into their individual components for qualitative and quantitative determinations. The simplicity of the technique permits its use by both chemists and trained technicians. Mass spectrometry, thin-layer chromatography, and ultraviolet and infrared spectrometries can all be used to complement the HPLC method and to aid in meeting the challenge of over-the-counter drug analysis.

References

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