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Mass spectrometry-compatible ICH (International Conference on Harmonization) impurity analysis with a high-pH mobile phase Advantages and pitfalls

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Abstract

Recent advances in bonding chemistry and novel silica synthesis have significantly extended the pH range of silica-based HPLC columns. This extended range now enables the analysis of water-soluble basic drugs at high pH without ion-pairing reagents, thus offering an alternative approach to assay or impurity analyses. This paper describes the many advantages and potential pitfalls of using high-pH mobile phases in the development of MS-friendly LC gradient impurity analytical methods for water-soluble basic drugs under International Conference on Harmonization (ICH) guidelines. Operating at high-pH provides excellent peak shapes and retention, and accentuates selectivity differences of structurally similar impurities and degradants. However, several problems unique to the use of high-pH mobile phases, such as column lifetime, robustness of pH adjustments, peak fronting, and on-column dimerization, were encountered. Each of these problems is discussed with its respective remedy.

Keywords: Pharmaceutical analysis; Mobile phase composition; Mass spectrometry compatibility; pH effects

1. Introduction

HPLC method development for impurity analysis of pharmaceuticals is a common but challenging task. According to International Conference on Harmonisation (ICH) guidelines [1], validated analytical methods of drug products should demonstrate separation of degradants from drug substance process impurities. This is particularly difficult to achieve for combination drug products with two or more active pharmaceutical ingredients (APIs) where all degradation products must be separated from each other, parents, excipients and other process impurities of the APIs. There is a growing trend towards gradient analysis (to increase peak capacity and better analysis of polar impurities and late-eluting dimers), photodiode array detection (for peak tracking and confirmation), and MS-compatible methodology (for easier identification of new impurities or degradants) [2,3].

For acidic or basic analytes, the mobile phase pH is a controlling factor for peak resolution. However, use of pH >8 has not been feasible for silica-based columns due to silica dissolution. While base-resistant packing materials such as polymers or zirconia are available, they are not widely used due to lower column efficiency, selectivity differences, or general unfamiliarity [4,5]. Recent advances [6–8] have dramatically increased the pH range of silica-based columns from the normal operating range of 2–7.5 to a wider range of 1–12; thus, offering a feasible

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approach for the analysis of water-soluble bases (as free bases) without ion-pairing reagents. This paper describes the advantages and pitfalls of this approach in the development an impurity method for basic drugs.

2. Experimental

2.1. Chemicals and reagents

ACS-grade reagents (ammonium hydroxide, phosphoric acid, potassium monobasic phosphate) and HPLC-grade solvents (acetonitrile, methanol, ethanol) were obtained from J.T. Baker (Phillipsburg, NJ, USA). Ammonium carbonate (99.999% purity) was obtained from Aldrich (Milwaukee, WI, USA).

2.2. Equipment

A Waters Alliance HPLC system equipped with a 2695 separations module, a column oven, an on-line vacuum degasser, a 996 photodiode array detector, and a 2487 absorbance detector was used. Both system control and data handling were performed by a Waters Millennium^{32} Client/server system. HPLC columns used were Waters XTerra columns (3.0 x150 mm) packed with 3.5-μm MS C_{18} or RP_{18} bonded phases. All HPLC systems and columns were obtained from Waters (Milford, MA, USA). For LC–MS analysis, a Waters Alliance system with an XTerra column (2.1×150 mm) was coupled to a ThermoFinnigan LCQ Classics ion trap system (ThermoFinnigan, San Jose, CA, USA) using an electrospray ionization interface and operated in the positive ionization mode.

2.3. Mobile phase A preparation

First, 1.6±0.1 g of ammonium carbonate was transferred into 1 l of HPLC grade water and mixed well. A small portion was poured into a separate beaker for pH testing to avoid dipping the pH electrode in the bulk solution. The pH of the buffer was adjusted to 9.10–9.15 using ammonium hydroxide. To reduce the chance of contamination through the filtration process, this mobile phase was not filtered.

2.4. HPLC conditions

The following optimized HPLC conditions were used for method validation and analysis of tablet extracts. Column: Waters XTerra RP_{18} column; Mobile phase: (A) 0.16% ammonium carbonate buffer at pH 9.10–9.15, (B) acetonitrile; gradient program: 2–60% B in 25 min, linear gradient; flow-rate: 0.7 ml/min at 40 °C; detection wavelength: 280 nm; run time: 35 min.

3. Results and discussion

3.1. Advantages of high-pH separations

Figs. 1 and 2 show chromatograms of a pharmaceutical tablet extract using the traditional isocratic ion-pair chromatography (Fig. 1) versus the alternate approach of high-pH gradient analysis (Fig. 2). The dramatic improvements shown in Fig. 2 are attributable to differences in the column and operating conditions. Note that the elution order of the two APIs was reversed from Fig. 1 to Fig. 2, and excellent peak shapes and resolution were obtained in Fig. 2. We believe that better overall resolution was due to higher column efficiency (n >15 000 plates), gradient elution, and operation at pH values close to pK_a of the APIs, where selectivity differences were accentuated between structurally similar components. The advantages of the high-pH gradient separation are:

(1) Retention of soluble bases without ion pairing

(2) Excellent chromatographic performance

(i) Good peak shape (tailing factors <1.1) due to reduced interaction of the unionized analytes with silanol groups and better analyte mass transfer without ion-pairing

(ii) Precision of retention time and peak area of APIs <0.5 and <0.2% of RSD, respectively

(iii) Limits of quantitation (LOQs) typically 0.01–0.02% of the parent

(3) Good peak resolution at optimum mobile phase pH

(4) MS compatibility allowing easier identification of impurities/degradants

Fig. 3 shows the retention time plot of several close-eluting components at mobile phase pH values

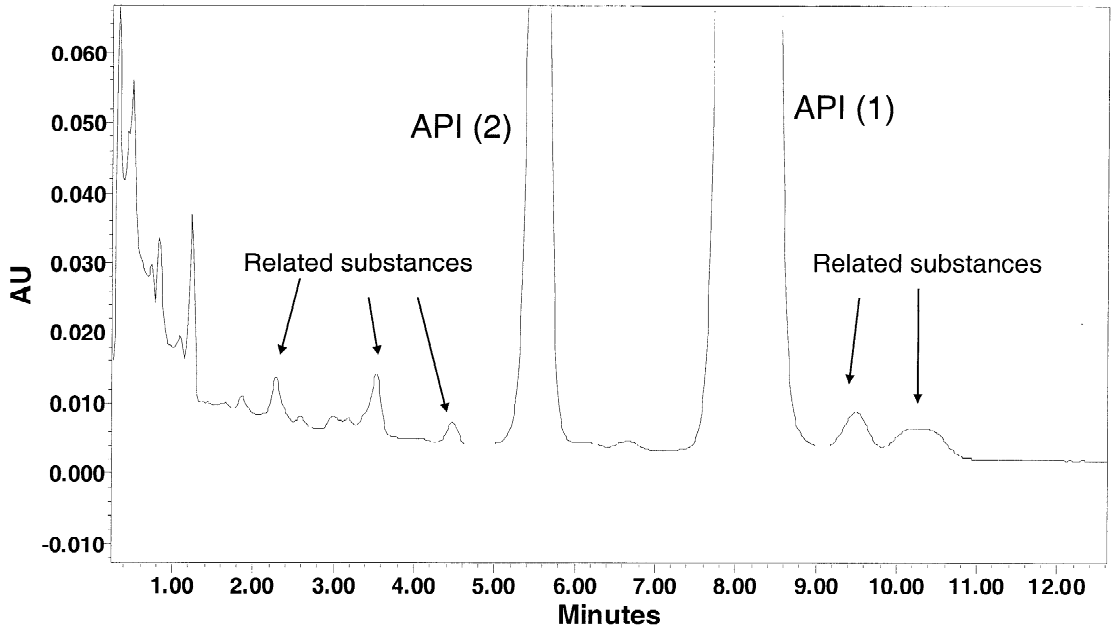

Fig. 1. Typical performance using traditional approach of isocratic ion-pairing HPLC of a tablet extract. HPLC conditions were: Waters Symmetry Shield RP_{18} column (4.6×50 mm, 3.5 μm), mobile phase: 0.43% sodium dodecyl sulfate in acetonitrile–water (20:80, v/v), pH 3.0, 2.5 ml/min 60 °C, 203 nm. These conditions are not MS-compatible due to the use of non-volatile ion-pairing reagent. API (1) and API (2) refer to the first and second active pharmaceutical ingredients, respectively.

in the range of 9.06–9.22, illustrating the importance of tight pH control to prevent peak coelution.

3.2. Potential pitfalls

During method development, numerous pitfalls or potential problem areas, were encountered. While many were attributed to typical trace analysis or gradient problems, several were unique to the high-pH approach. Fortunately, most can be avoided or remedied through judicious operation. These pitfalls are described below.

3.2.1. Column lifetime

The wide pH range of 1–12 quoted by the manufacturer for using XTerra MS C_{18} columns is attributed to the hybrid particle and the poly-functional silane bonding chemistry [8]. The XTerra RP_{18} particle with the polar embedded group has a narrower pH stability range of 2–12. Note that these pH ranges are quoted for room temperatures and the pH stability range is substantially reduced at elevated temperatures.

We found typical column lifetimes for XTerra RP_{18} columns to be about 3 months and >1000 injections under our experimental conditions of pH 9 and 40 °C. We did, however, experienced an early column failure by operating overnight at pH 10 and 40 °C.

3.2.2. Blank gradient issues

It is a common practice in impurity analysis to make a blank injection of the extraction solvent before conducting sample testing. Fig. 4 shows chromatograms comparing a ''good'' and a ''bad'' blank injection. Since the column is equilibrated initially with several milliliters of the buffer with low organic content, any trace impurities in mobile phase A are concentrated at the head of the column and elute as distinct gradient peaks. Meticulous care and cleanliness must be exercised in the preparation of mobile phase A. We selected a high-purity ammonium carbonate (99.999%) and were able to skip the membrane filtration step without any system problems. We often experienced spurious gradient peaks from contaminated HPLC systems. Rinsing solvent

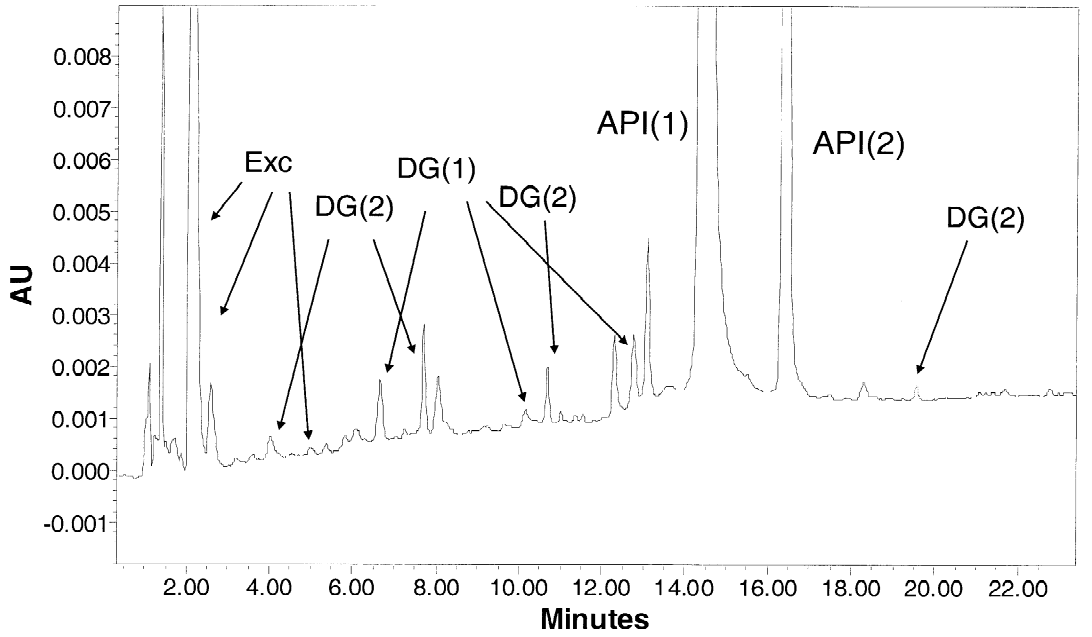

Fig. 2. Typical performance of gradient analysis of the tablet extract using high-pH mobile phase under conditions described in the experimental section. The small peak at 19.4 min is a dimer, which is not detectable under conditions in Fig. 1. These conditions are MS-compatible due to the use of volatile buffers. Exc=excipient, DG (1)=degradant from API (1), etc. Other unlabeled peaks are impurities.

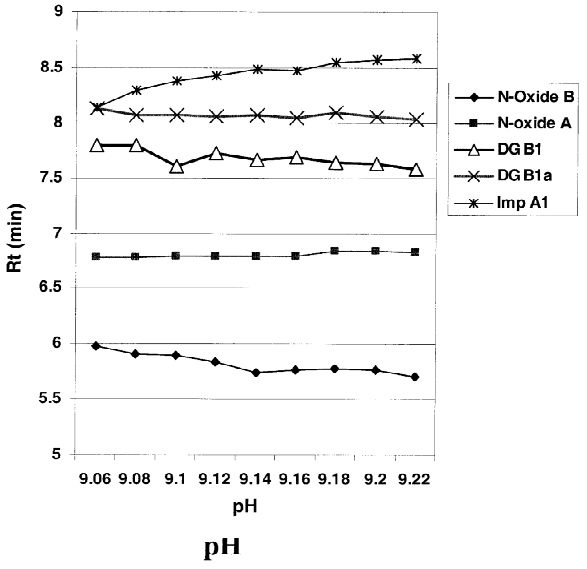

Fig. 3. Plot of retention times of several close-eluting components at mobile phase pH values in the range of 9.06–9.22.

lines and systems with water, organic solvents (even 6 *M* nitric acid in severe cases), often reduced the problems. For routine impurity testing, we suggest using dedicated HPLC systems.

During early method development, a substantial gradient peak was encountered in several buffer preparations (Fig. 5). Note that the contaminant peak eluted closely to the second API and was 10–100 times higher than typical gradient peaks shown in Fig. 4. Curiously, this peak was only found in buffer preparations requiring pH adjustment. This peak was eventually traced to an UV-absorbing preservative used in some pH calibration buffers [9] and was eliminated by not dipping the pH electrode in the mobile phase reservoir.

3.2.3. pH issues

One potential pitfall of operating at a mobile phase pH close to the pK_a of the API is the sensitivity of the separation to minor pH variations. For robust separations in our application, the pH of the mobile phase should be close to the narrow optimum target range of 9.10–9.15. To ensure accurate pH measure-

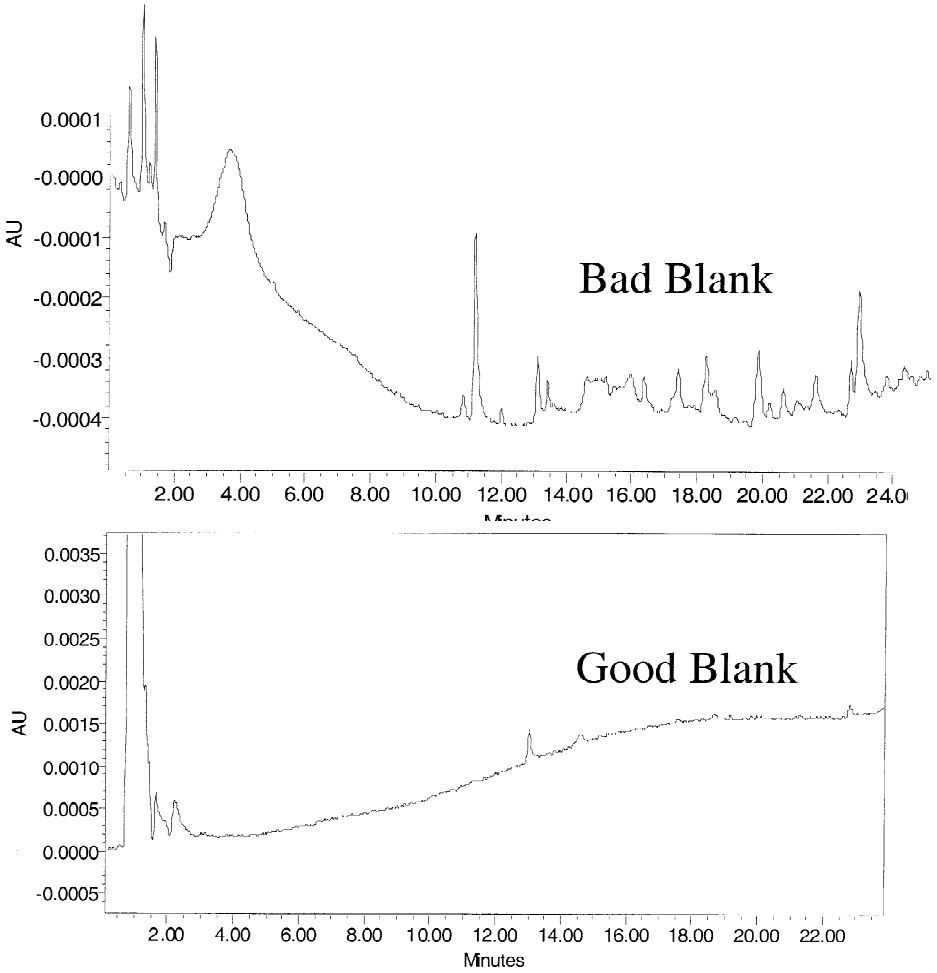

Fig. 4. Chromatograms comparing of a ''good'' and a ''bad'' blank injection of the extraction solvent.

ments, pH meters with automatic temperature compensation and properly maintained electrodes should be used and calibrated with fresh calibration buffers before use.

3.2.4. Robustness

Relative retention times (RRTs) are typically used in impurity test methods to identify known impurities and unspecified degradants. As it is not always practical to provide all impurity standards solutions for routine testing, correct peak identification by RRT is predicated on column-to-column consistency and other robustness issues. During method validation, we found excellent lot-to-lot consistency of the XTerra columns as shown in Fig. 6. RSDs of the 12 runs from four column lots and three pHs were typically $<1\%$. Another obvious robustness factor is the HPLC system dwell volumes, which should be less than one milliliter for this assay. In our method, we included a system suitability sample [10] of the APIs spiked with several key degradant standards at 0.1% levels to ensure correct identification of those peaks. We also recommended the use of a photodiode array detector and provided λ_{max} values for all specified degradants in the test method to prevent peak mis-identification.

3.2.5. Other issues

During method development, excellent peak shapes were generally observed. Peak fronting of the

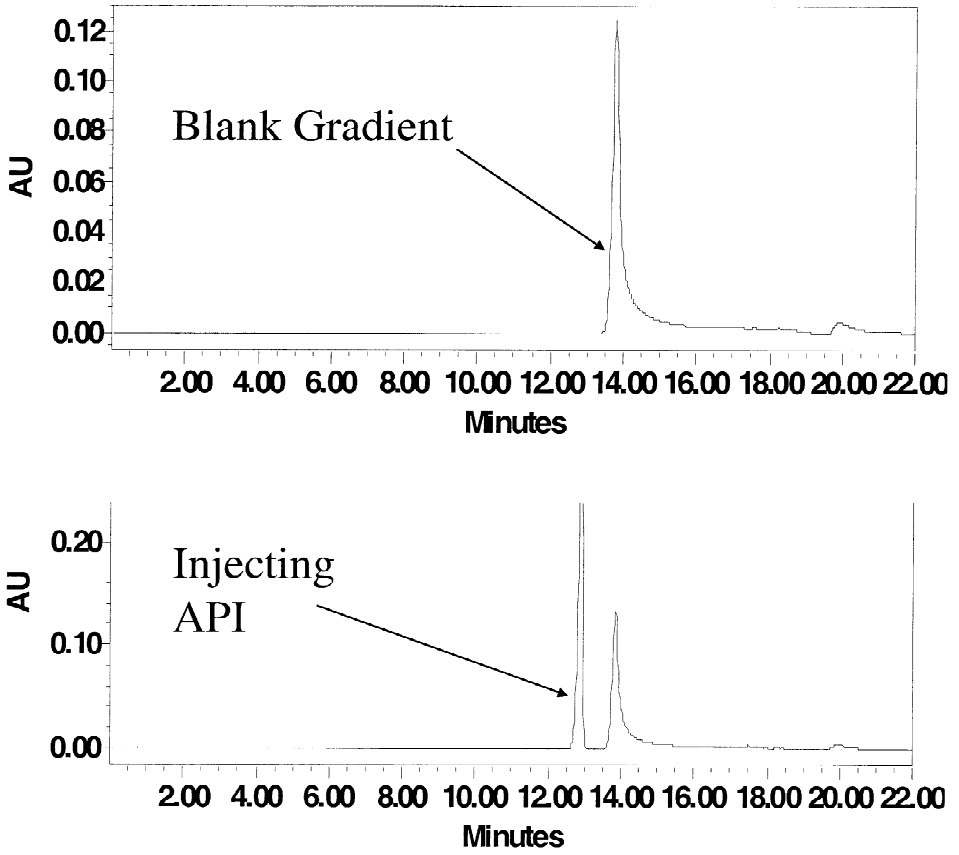

Fig. 5. Gradient peak stemming from the contamination of the mobile phase buffer with an UV-absorbing preservative from pH calibration buffers. The bottom chromatogram shows the relative retention time and peak size of the contaminant peak in comparison with the second API peak.

API occurred when the mobile phase buffering capacity was exceeded or when the pH was outside of the buffer range. We did not observe peak splitting or other anomalies due to the separation of the ionized and un-ionized forms of the same analyte. Two process impurities displayed peak broadening or splitting, which correlated with injection volume and solvent strength, and perhaps the presence of isomeric forms. Further future investigation is warranted.

We also found an increasing trend of higher assay values of a dimer peak eluting at 19.4 min from the same tablet sample within a 2-months period during early method development. We suspected that this dimer was formed on a particular column because substantial dimerization (~5%) was observed just by injecting a solution of pure API as shown in Fig. 7. Injecting the same API solution in another system with a different column showed a typical dimer level of $<0.05\%$. We finally traced this anomaly to on-column dimerization of the injected API, which was catalyzed by the gradual accumulation of trace metals (e.g. iron) at the head of the column. This phenomenon was not observed with acidic pHs since trace metals are soluble in acids. This problem was remedied by periodically washing the column with 0.5% acetic acid.

3.3. Summary and conclusions

High-pH mobile phases are feasible for impurity analysis of water-soluble basic drugs according to recent ICH guidelines. Advantages are ‘‘tunable’’ resolution by varying pH, excellent peak shapes and

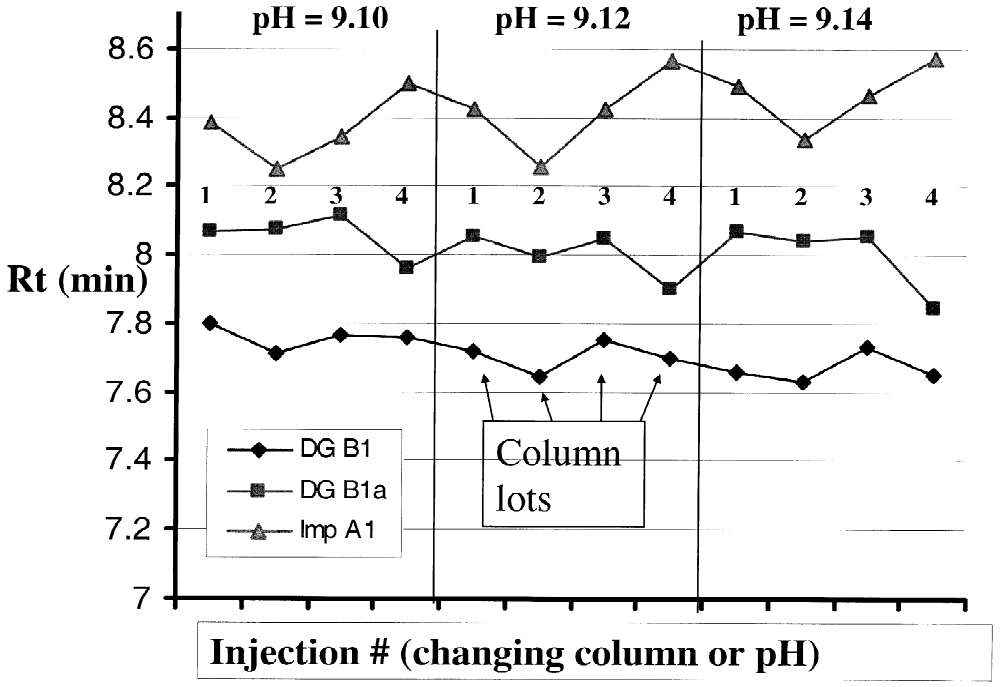

Fig. 6. Retention time plots of three close-eluting components using Xterra RP_{18} columns from four silica lots at three different pH values.

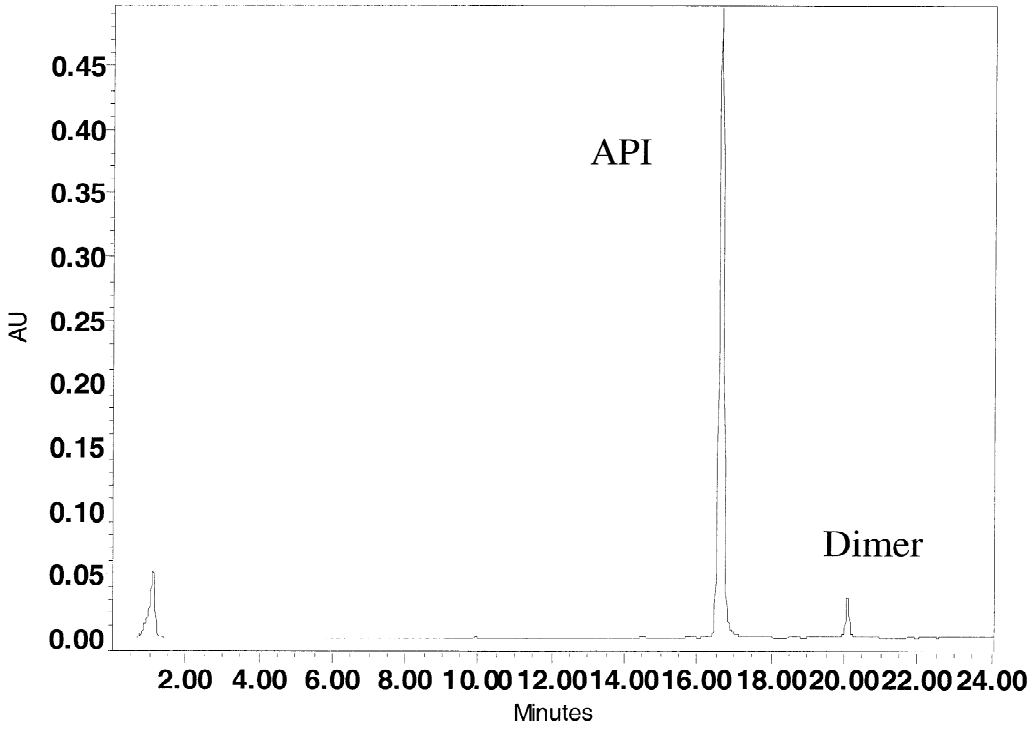

Fig. 7. Chromatogram of an injection of a pure API (2) solution showing the on-column formation of a dimer peak caused by trace metal contamination at the top of the column. This dimer peak disappeared after the column was rinsed with acidic solution.

sensitivity, and MS compatibility. Potential pitfalls are problems associated with: column lifetime, gradient blanks, pH adjustments, and robustness in peak identifications. Most problems can be overcome by judicious operations.

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