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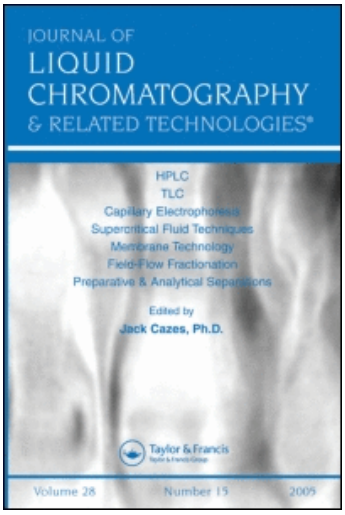

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
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Evaluation of Chromatographic Methods for Drug Products Containing Polar and Non-Polar Molecules Using Reversed Phase, Hydrophilic Interaction, and Ion Exchange Chromatography

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To cite this Article Rogers, Lance A. , Crews, Karen E. , Long, Susan G. , Patterson, Kristin M. and McCune, John E.(2009) 'Evaluation of Chromatographic Methods for Drug Products Containing Polar and Non-Polar Molecules Using Reversed Phase, Hydrophilic Interaction, and Ion Exchange Chromatography', Journal of Liquid Chromatography & Related Technologies, 32: 15, 2246 — 2264
To link to this Article: DOI: 10.1080/10826070903163057
URL: http://dx.doi.org/10.1080/10826070903163057

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Journal of Liquid Chromatography & Related Technologies®, 32: 2246–2264, 2009
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ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826070903163057

Evaluation of Chromatographic Methods for Drug Products Containing Polar and Non-Polar Molecules Using Reversed Phase, Hydrophilic Interaction, and Ion Exchange Chromatography

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Abstract: In recent years the pharmaceutical industry has developed an increasing variety of complex double or triple combination drug therapies. Method development for these combination drug products is particularly challenging when the analytes have significantly different polarities, often requiring multiple chromatographic methods for each active component. An alternative to the commonly used practice of developing multiple reversed phase chromatographic methods for analyzing small molecules in combination drug products is discussed herein.

Ion exchange chromatography offers many advantages to other chromatographic techniques for the analysis of combination products if one of the molecules is highly polar ($\log P < 0$) and the other moderately polar to non-polar ($\log P \geq 0$). As the primary mode of retention for ion exchange chromatography is based upon the charge of the molecule, the hydrophobicity of the molecule does not play as large of a part in the retention mechanism. As long as all of the molecules being analyzed are ionizable, retention on an ion exchange column should be possible. The analysis of polar and non-polar molecules simultaneously using ion exchange chromatography is a unique process, and the approach of developing accurate and robust analytical methods for analysis of all active pharmaceutical ingredients in a combination product by a single fast ion exchange method differs from the industry standard of using reverse phase methodology first.

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Six well characterized pharmaceuticals with varying degrees of polarity (metformin HCl, isoniazid, sodium nitroprusside, timolol maleate, naproxen sodium, and clonidine hydrochloride) have been chromatographically evaluated using RP, HILIC, and ion exchange chromatography. In addition, a well characterized pharmaceutical product, Metaglip™ Tablets, containing the highly polar molecule metformin HCl and the moderately non-polar glipizide, has been analyzed using a cation exchange (CE) HPLC method and compared to chromatography and results generated using RP-HPLC and hydrophilic interaction (HILIC)-HPLC.

Keywords: Drug products, Hydrophilic interaction, Ion chromatography, Non-polar, Polar, Reversed Phase

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INTRODUCTION

By far, the most common mode of chromatographic separation used in the pharmaceutical industry is reversed phase high performance liquid chromatography (RP-HPLC). Over the past two decades the increase in diverse, as well as robust, reverse phase columns has often lead to their use as a ''first intent'' platform for content methods.[1] The analysis of combination products containing two or more active pharmaceutical ingredients (API's) places high demands on RP-HPLC analysis in regard to retention of the analytes, resolution from impurities and retained excipients or other active components, peak shape, and column robustness.

The polarity and ability of a molecule to be ionized greatly influence its retention on an organic RP-HPLC column. If the API's in a combination drug product have significantly differing polarities, the analyst will often be forced to develop multiple chromatographic methods. Development and subsequent validation of multiple methods is time and resource consuming.

Analysis of combination drug products has also been well documented, although by far, the most common means of their analysis has been using RP-HPLC.[2] However, the use of alternative modes of chromatographic separation may be more appropriate for the analysis of combination products. Many papers have been published describing the analysis of individual polar and moderately non-polar molecules using chromatographic methods such as reverse phase,[3–5] normal phase,[6,7] ion-pair,[8,9] ion exchange,[10,11] and hydrophilic interaction chromatography (HILIC).[12–15]

HILIC, although not as well known or commonly used, has advantages over RP-HPLC in that both acidic and basic polar molecules can be retained. Strong retention of polar molecules is possible and adjustments to mobile phase composition can greatly influence the desorption of analyte molecules.

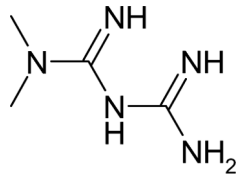

Figure 1. Structure of metformin.

Ion exchange chromatography offers many advantages to other chromatographic techniques for the analysis of combination products if one of the molecules is highly polar ($\log P < 0$) and the other moderately polar to non-polar ($\log P \geq 0$). As the primary mode of retention for ion exchange chromatography is based upon the charge of the molecule, the hydrophobicity of the molecule does not play as large of a part in the retention mechanism. As long as all of the molecules being analyzed are ionizable, retention on an ion exchange column should be possible. The analysis of polar and non-polar molecules simultaneously using ion exchange chromatography is a unique process, and the approach of developing accurate and robust analytical methods for analysis of all active pharmaceutical ingredients in a combination product by a single fast ion exchange method differs from the industry standard of using reverse phase methodology first.

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Seven well characterized pharmaceuticals: metformin (Figure 1), glipizide (Figure 2), isoniazid (Figure 3), clonidine (Figure 4), timolol (Figure 5), naproxen (Figure 6), and sodium nitroprusside (Figure 7) have been chromatographically evaluated using RP, HILIC, and ion exchange (cation exchange or anion exchange) chromatography.

Metformin, isoniazid, and sodium nitroprusside are highly polar ($\log P < 0$). Sodium nitroprusside is an acid and both metformin and isoniazid are bases. Timolol, naproxen, glipizide, and clonidine are moderately non-polar ($\log P > 0$). Naproxen is an acid while timolol, glipizide, and clonidine are bases. The retention, peak shape, and efficiency

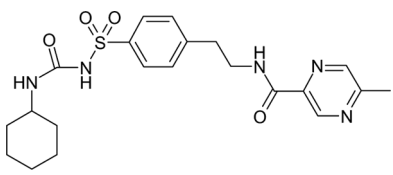

Figure 2. Structure of glipizide.

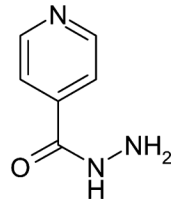

Figure 3. Structure of isoniazid.

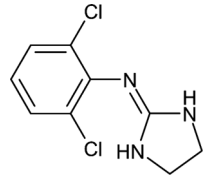

Figure 4. Structure of clonidine.

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have been qualitatively evaluated and are compared for all molecules using the three chromatographic techniques.

In addition, a well characterized pharmaceutical product, Metaglip™ Tablets, containing the highly polar molecule metformin HCl and the moderately non-polar glipizide, has been analyzed using a cation exchange (CE) HPLC method and compared to chromatography and

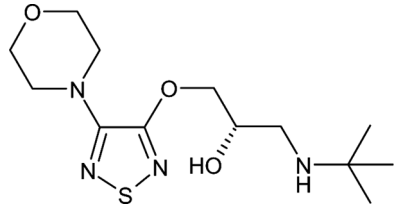

Figure 5. Structure of timolol.

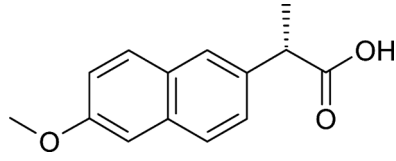

Figure 6. Structure of naproxen.

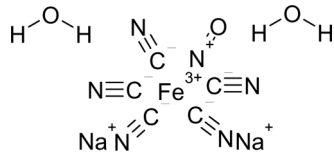

Figure 7. Structure of sodium nitroprusside.

results generated using RP-HPLC and hydrophilic interaction (HILIC)-HPLC.

EXPERIMENTAL

Materials

Metformin HCl and naproxen sodium were obtained from Glaxo SmithKline. Isoniazid, sodium nitroprusside, timolol maleate, and clonidine HCl were obtained from VWR. Metaglip™ Tablets were obtained from Bristol-Myers Squibb.

Reagents

Reagents used were calcium phosphate monobasic (95%, Sigma), acetonitrile (HPLC grade, Burdick and Jackson), ammonium formate (99%, Acros), formic acid (98%, J. T. Baker), trifluoroacetic acid (99.5%, J. T. Baker), methanol (HPLC grade, Burdick and Jackson), deionized water, sodium phosphate dibasic (99.7%, J.T. Baker), phosphoric acid (85%, Fluka), and hydrochloric acid (36.5–38.0%, J. T. Baker).

Instrumentation

The HPLC system used was an Agilent HP1100 with a G-1311A pump, G1322A degasser, G1313A autosampler, G1316A column heater, and G1314A variable wavelength detector. Data was collected using Atlas (Thermo Labsystems).

Sample Preparation

Solutions of each individual compound (metformin, isoniazid, sodium nitroprusside, timolol, naproxen, and clonidine) were prepared at approximately 0.15 mg/mL in 0.1 N HCl:Acetonitrile (50:50).

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Table 1. Log P (octanol/water) and pKa for listed compounds

Compound	$\text{Log P}_{(o/w)}$	pKa	Acid/Base
Isoniazid	−0.8	1.8	base
Metformin HCl	−2.64	2.9, 11.5	base
Sodium nitroprusside	n/a	n/a	acid
Clonidine HCl	1.57	8.2	base
Glipizide	2.5	5.9	base
Naproxen Na	3.24	4.2	acid
Timolol maleate	2.12	9.5	base

A solution of Metaglip™ Tablets containing glipizide (2.5 mg per tablet) and metformin HCl (500 mg per tablet) was prepared at a concentration of 0.0025 mg/mL glipizide and 0.5 mg/mL metformin HCl in 0.1 N HCl:Acetonitrile (50:50).

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Chromatographic Conditions

Reverse Phase Chromatography (RP-HPLC) Conditions

A fast RP-HPLC method was used for the analysis of all samples. The method that was used for this paper is a typical screening method used for the analysis of API's in early pharmaceutical development and employs a gradient from low organic mobile phase concentration to high organic concentration over a short period of time. The aqueous mobile phase has been modified with a strong acid to act as an ion pairing agent for basic compounds and to minimize extra column retention mechanisms.

The column used for reversed phase analysis was a Zorbax SB-C_8 column (4.6 × 150 mm, 3.5 µm particle size). A C_8 column was chosen over a C_{18} to attempt to maximize the retention of more polar molecules using reverse phase chromatography. Elution was accomplished using trifluoroacetic acid (TFA) as an ion pairing agent (0.05%, v/v in water) in the aqueous mobile phase (mobile phase A, MPA) and 0.05% v/v TFA in acetonitrile as the organic mobile phase (mobile phase B, MPB). Chromatographic conditions can be found in Table 2.

Hydrophilic Interaction Chromatography (HILIC) Conditions

HILIC chromatography is a variation of normal phase chromatography where a polar stationary phase is used with an aqueous polar solvent. HILIC columns are typically used to retain and separate polar, water soluble, basic organic compounds.

Table 2. Reverse phase HPLC chromatography parameters

Time	Mobile phase A(%)	Mobile phase B(%)	Flow (mL/min)	Column temp. (°C)
0.00	90	10	1.5	40
2.00	90	10		
8.00	10	90		
8.10	90	10		
10.0	90	10		

A HILIC method was created for analysis of all samples. A very shallow gradient was used in an attempt to retain less polar molecules while allowing more polar molecules to elute in a reasonable amount of time. A salt was chosen that would act as an ion suppressor yet stay soluble in highly concentrated organic mobile phases.

A Waters Atlantis® HILIC column (2.1 × 50 mm, 3 µm particle size) was used for hydrophilic interaction analysis. Elution was accomplished using 100% acetonitrile (MPA) as the organic mobile phase and a 25 mM ammonium formate buffer at pH 4.2 (adjusted with formic acid) as the aqueous mobile phase (MPB). Chromatographic conditions can be found in Table 3.

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Cation Exchange Chromatography (CX-HPLC) Conditions

Ion exchange chromatography allows the separation of ions or polar molecules based on their charge. Cation exchange resins contain a negatively charged functional group, which will retain positively charged cations.

A generic isocratic cation exchange method was developed for the analysis of basic samples. Consideration had to be given to the choice

Table 3. Hydrophilic interaction chromatography parameters

Time	Mobile phase A(%)	Mobile phase B(%)	Flow (mL/min)	Column temp. (°C)
0.00	95	5	1.0	40
12.00	90	10		
12.10	95	5		
15.00	95	5		

Table 4. Cation exchange HPLC chromatography parameters

Time	Mobile phase A(%)	Mobile phase B(%)	Flow (mL/min)	Column temp. (°C)
0.00	75	25	3.0	40
5.00	75	25		

and strength of the exchange ion due to the greatly varying retention times of the basic molecules. Metformin is highly retained on a strong cation exchange column and isoniazid is slightly retained. Metformin would not elute with a weak exchange ion (Na^+ and K^+) at reasonable concentrations and required calcium (Ca^{2+}) as the exchange cation. The other basic compounds would elute with sodium and potassium; however, to maintain a fast LC method the stronger exchange ion calcium was chosen. Additionally, the retention of metformin is not greatly influenced by changing the concentration of organic whereas the retention time of other more polar molecules can be manipulated by changes in the organic concentration.

A strong cation exchange column was chosen for analysis of ionizable bases (Agilent Zorbax 300-SCX analytical column, 4.6×150 mm, 5 μm particle size). Elution was accomplished using 0.01 M calcium phosphate monobasic, at pH 2.5 (adjusted with HCl) (MPA) and 100% methanol (MPB). Chromatographic conditions can be found in Table 4.

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Anion Exchange Chromatography (AX-HPLC) Conditions

Anion exchange resins contain a positively charged functional group, which will retain negatively charged anions.

A simple gradient anion exchange method was developed for the analysis of acidic samples. The initial mobile phase consisted of moderate strength exchange ions at low concentration, ramping to a higher ionic concentration throughout the run.

A strong anion exchange column was chosen for analysis of the ionizable acidic compounds (Agilent Zorbax SAX analytical column, 4.6×150 mm, 5 μm particle size). Phosphate was chosen as the exchange anion due to the strength of the ion relative to the strength of the analytes. Elution was accomplished using 0.01 M sodium phosphate dibasic, pH 6.1, adjusted with phosphoric acid (MPA), 0.1 M sodium phosphate dibasic, pH 6.3, adjusted with phosphoric acid (MPB) and 100% methanol (MPC). Chromatographic conditions can be found in Table 5.

Table 5. Anion exchange HPLC chromatography parameters

Time	Mobile phase A(%)	Mobile phase B(%)	Mobile phase C(%)	Flow (mL/min)	Column temp. (°C)
0.00	70	0	30	3.0	40
2.00	70	0	30		
8.00	0	70	30		
8.10	70	0	30		
10.00	70	0	30		

RESULTS AND DISCUSSION

RESULTS

Chromatographic Results for Basic Compounds

RP-HPLC Chromatography. Reversed phase analysis of the non-polar basic compounds timolol maleate and clonidine HCl (Figure 8) revealed acceptable retention ($k' > 1.0$) for both. Peak shape and efficiency were acceptable for these compounds as well (Table 6), however; the polar compounds, metformin and isoniazid were poorly retained ($k' = 0$) by the C_8 column using the chromatographic conditions described.

The Metaglip^{TM} combination drug product was analyzed using the RP-HPLC method. As shown in Figure 9 and Table 7, acceptable retention, peak shape, and column efficiency were achieved for the moderately non-polar glipizide, however, the highly polar metformin was not retained.

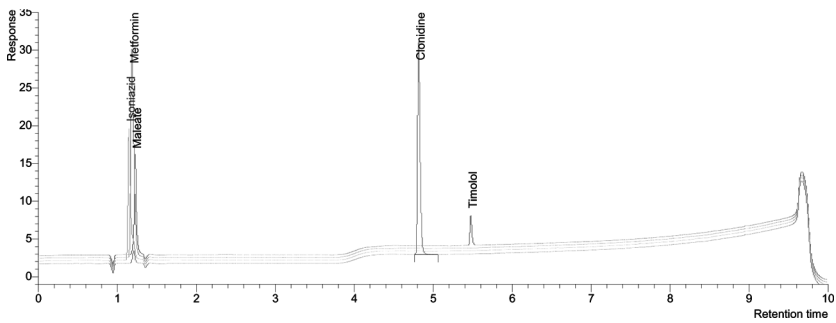

Figure 8. Chromatograms of basic compounds by reverse phase HPLC.

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Table 6. Chromatographic results for analysis of basic compounds by reverse phase HPLC

	Column efficiency (N_{base}, USP)	Tailing factor (T, USP)	Capacity factor (k', USP)
Maleate counter-ion (Timolol)	ND	ND	0
Metformin	ND	ND	0
Isoniazid	ND	ND	0
Clonidine	105716	1.4	3.0
Timolol	246135	1.3	3.6

ND – not determined.

Hydrophilic Interaction Chromatography (HILIC)

Analysis by hydrophilic interaction chromatography revealed acceptable chromatography (Figure 10). Retention and peak shape of bases metformin, timolol, clonidine, and isoniazid were acceptable (Table 8). Metformin, which is the most polar of the four bases is most strongly retained, while isoniazid and clonidine, the more non-polar bases, are not as well retained.

Cation Exchange (CX) Chromatography

Analysis by cation exchange chromatography revealed acceptable retention of all basic compounds (Figure 11). Peak shape and efficiency were acceptable in all cases (Table 9). The use of cation exchange chromatography yields an efficient methodology for content analysis of combined products of non-polar and polar basic compounds.

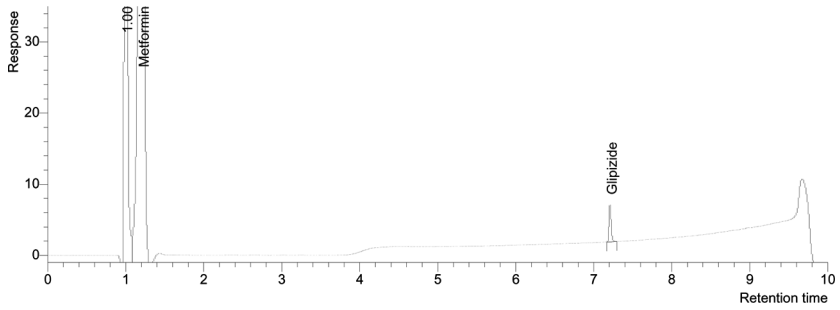

Figure 9. Chromatogram of combination drug product Metaglip™ containing glipizide and metformin HCl by reverse phase HPLC.

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Table 7. Chromatographic results for analysis of combination drug product Metaglip™ containing glipizide and metformin by reverse phase HPLC

	Column efficiency (N_{base}, USP)	Tailing factor (T, USP)	Capacity factor (k', USP)
Metformin	ND	ND	0
Glipizide	344039	1.2	7.6

ND – not determined.

Analysis of the combination tablet Metaglip™ demonstrates that both the polar and non-polar molecules are well retained (Figure 12). Peak shape and efficiency were acceptable for both glipizide and metformin (Table 10).

Chromatographic Results for Acidic Compounds

RP-HPLC Chromatography

Analysis by reverse phase HPLC revealed acceptable retention of the non-polar acidic compound naproxen ($k' > 1.0$) (Figure 13). Peak shape and efficiency were acceptable for this compound as well (Table 11); however, the polar acidic compound sodium nitroprusside was poorly retained by the C_8 column ($k' \sim 0$).

Hydrophilic Interaction Chromatography (HILIC)

No retention was observed for the polar compound nitroprusside or the non-polar compound naproxen when analyzed by hydrophilic interaction chromatography (Figure 14). Lack of retention of acids on a HILIC column using the mobile phases and conditions described in this paper were expected.

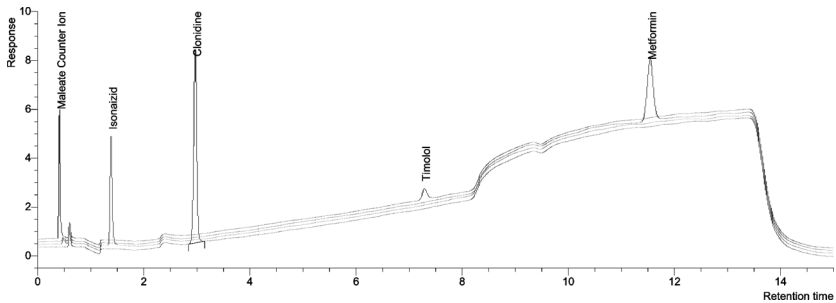

Figure 10. Chromatograms of basic compounds by hydrophilic interaction chromatography.

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Table 8. Chromatographic results for analysis of basic compounds by hydrophilic interaction chromatography

	Column efficiency (N_{base}, USP)	Tailing factor (T, USP)	Capacity factor (k', USP)
Maleate counter-ion (Timolol)	ND	ND	0
Isoniazid	6136	1.2	1.8
Clonidine	12419	0.9	5.0
Timolol	31620	1.1	13.6
Metformin	47186	1.0	22.1

ND – not determined.

Anion Exchange (AX) Chromatography

Analysis by anion exchange chromatography revealed acceptable retention of acidic compounds, naproxen and sodium nitroprusside (Figure 15). Peak shape and efficiency were acceptable for both compounds (Table 12). The basic compound timolol maleate was also analyzed by anion exchange chromatography to determine the retention and peak shape of the maleate counter ion. Maleate was retained well and also had acceptable peak shape and efficiency. As expected, timolol, a base, was not retained.

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DISCUSSION

RP-HPLC Chromatography

Analyses of polar and non-polar compounds were successfully completed using several HPLC techniques. RP-HPLC, which is the most common

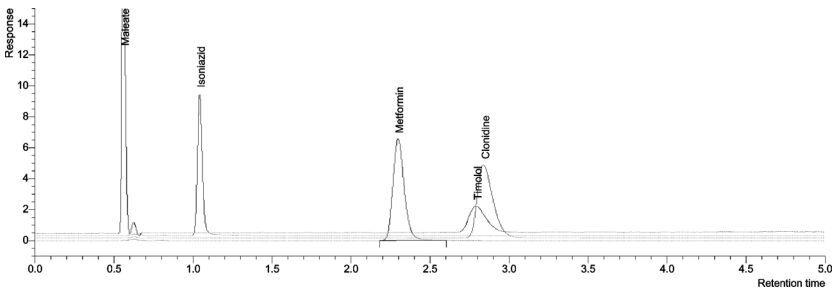

Figure 11. Chromatograms of basic compounds by cation exchange HPLC.

Table 9. Chromatographic results for analysis of basic compounds by cation exchange HPLC

	Column efficiency (N_{base}, USP)	Tailing factor (T, USP)	Capacity factor (k', USP)
Maleate counter-ion (Timolol)	ND	ND	0
Isoniazid	5685	1.2	1.1
Metformin	5082	1.2	3.6
Timolol	2837	1.3	4.6
Clonidine	3872	1.4	4.7

ND – Not determined.

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chromatographic technique used in the pharmaceutical industry for the analysis of small molecules, successfully retained all of the acidic and basic non-polar compounds (clonidine, glipizide, timolol, and naproxen). The peak shape was adequate and column efficiency was very high. Consideration of the quick gradient ramp (6 minutes) from low to high organic must be made when evaluating the high column efficiency. Although the column efficiency appears to be high, the quick gradient ramp with a drastic change in mobile phase composition will tend to decrease resolution of potential impurities with similar organic structure to the API, which can impact specificity of the method. Lengthening the gradient time will tend to resolve coeluting or closely eluting impurities, but will drastically reduce column efficiency while increasing retention time and tailing factor.

RP-HPLC is not acceptable for the analysis of acidic or basic polar molecules due to the lack of retention. The retention of metformin, isoniazid, and sodium nitroprusside was either in or before the void volume. Analysis of Metaglip™ Tablets also demonstrated that RP-HPLC is not

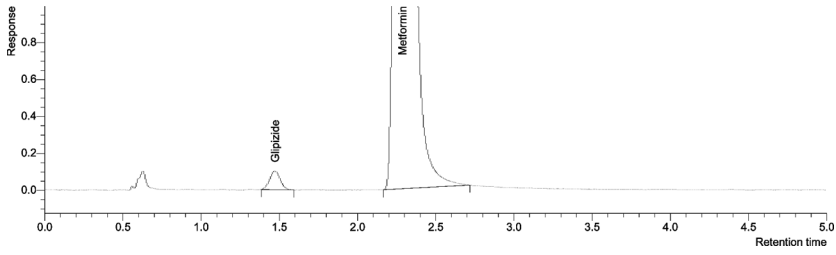

Figure 12. Chromatogram of combination drug product Metaglip™ containing glipizide and metformin HCl by cation exchange HPLC.

Table 10. Chromatographic results for analysis of combination drug product Metaglip™ containing glipizide and metformin by cation exchange HPLC

	Column efficiency (N_{base}, USP)	Tailing factor (T, USP)	Capacity factor (k', USP)
Glipizide	2262	1.1	1.9
Metformin	5080	1.3	3.6

acceptable for mixtures of polar (metformin) and non-polar (glipizide) compounds. Although glipizide was acceptably retained, there was no retention of metformin.

Modifications to the RP-HPLC method can be made to increase retention of polar molecules; however, by using the most common practices of including strong ion pairing agents (i.e., trifluoroacetic acid, SLS, methylsulfonic acid) and other additives, the robustness of the method can greatly be compromised. Only marginal chromatographic improvement would then be obtained.

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HILIC Chromatography

Hydrophilic interaction chromatography was successful in retaining the polar basic compounds metformin and isoniazid and the non-polar basic molecules clonidine and timolol ($k' \geq 1.0$). The tailing factor was acceptable for all molecules. Retention of the acidic molecules was not obtained using the method conditions. Retention of polar acidic molecules should be possible by changing method conditions to include higher pH mobile phase; however, development of additional HILIC methods is beyond the scope of this paper. The column efficiency was acceptable for all four basic compounds.

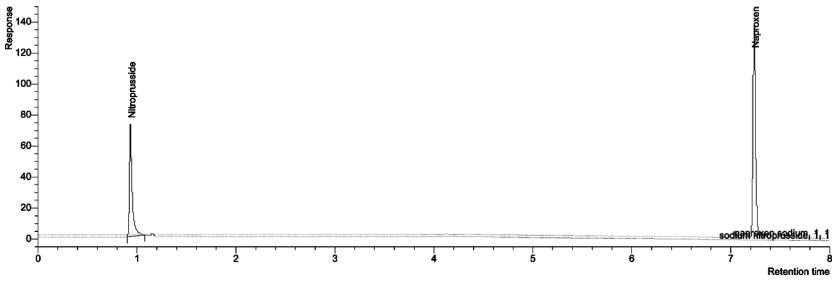

Figure 13. Chromatograms of acidic compounds by reverse phase HPLC.

Table 11. Chromatographic results for analysis of acidic compounds by reverse phase HPLC

	Column efficiency (N_{base}, USP)	Tailing factor (T, USP)	Capacity factor (k', USP)
Sodium Nitroprusside	ND	ND	0
Naproxen	354003	1.2	5.0

ND – not determined.

Hydrophilic interaction chromatography, although having been used for over 20 years,[16] is not as well known or commonly used in the industry as other chromatographic techniques; however, it offers an interesting yet viable alternative to more widely used chromatographic techniques, such as normal phase chromatography, for the retention of polar molecules.

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Cation Exchange Chromatography

Cation exchange chromatography was successful for the retention of both basic polar molecules metformin and isoniazid and the less-polar bases timolol and clonidine ($k' > 1.0$). The peak shape and column efficiency for all molecules was very good. An injection of the combination drug product Metaglip™ demonstrates that both compounds are well resolved and show no deleterious effects from drug product excipients when analyzed together. Improved chromatography and increasing resolving power for potential impurities and excipients that could coelute with the main components could be achieved by changing several method parameters. Examples are such as reducing the strength of the cation

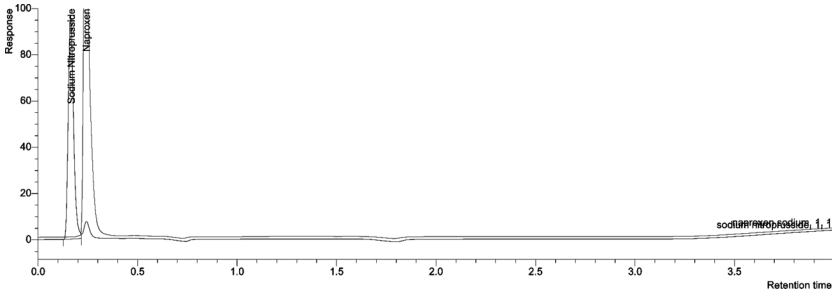

Figure 14. Chromatograms of acidic compounds by hydrophilic interaction chromatography.

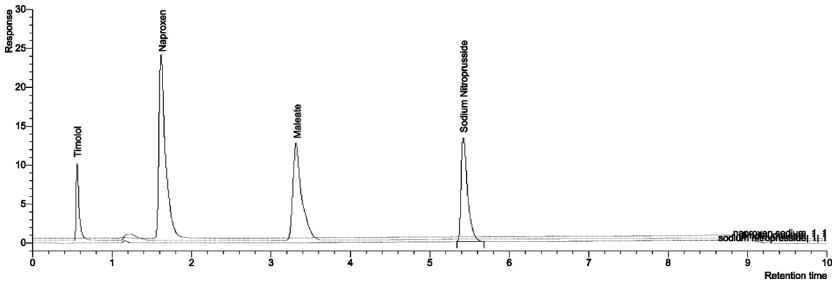

Figure 15. Chromatograms of acidic compounds by anion exchange HPLC.

found in mobile phase A, creating a gradient of low to high concentration of an exchange ion, creating a gradient of increasing strength of exchange ions (e.g., ramping from Na^+ to Ca^+), and changing pH. The flexibility of making multiple changes to the mobile phase simultaneously is one of the benefits of ion exchange over reversed phase chromatography. With RP-HPLC the three major changes that will result in the potentially greatest retention and resolution of retained molecules are change in organic solvent, change in organic solvent concentration (organic and aqueous mobile phase ratios), and change in pH of the aqueous mobile phase. However, these changes are not viable solutions to improving retention time of polar compounds when using RP-HPLC. With ion exchange chromatography several mobile phase factors can be varied to give extreme resolving and retention mechanisms within the same run. Although beyond the scope of this paper, development and use of such methods should be viable.

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Anion Exchange Chromatography

Anion exchange chromatography was successful for the retention of the acidic polar molecule nitroprusside, as well as the acidic non-polar

Table 12. Chromatographic results for analysis of acidic compounds by anion exchange HPLC

	Column efficiency (N_{base}, USP)	Tailing factor (T, USP)	Capacity factor (k', USP)
Timolol	ND	ND	0
Naproxen	2036	2.1	1.7
Maleate counter-ion (Timolol)	5125	1.8	4.5
Sodium Nitroprusside	22782	1.8	8.2

ND – not determined.

molecule naproxen and the timolol counter ion maleate. The retention time was acceptable for all; however, some tailing was noted. Changes to the mobile phase, including decreasing the strength/concentration of the ion in mobile phase A, or slight changes to the HPLC conditions should be able to improve peak shape, increase retention, and increase column efficiency.

CONCLUSION

This paper demonstrates the ability of several different HPLC methods to retain individual polar and non-polar molecules. Although reverse phase chromatography is often the most commonly used mode of chromatographic separation, several additional modes of separation exist that may be better suited for chromatographic analysis of combined molecules. As the complexity of the pharmacological molecules and the formulations for drug delivery increase, thought must be given to using the most efficient technique that will result in good retention, resolution, peak shape, and method robustness. This is especially true when considering combination drug products.

Method development often commences with consideration of the molecule polarity when developing a reverse phase method. When analyzing combination drug products, a common practice is to develop multiple methods for the analysis of all APIs. This is demonstrated in the USP methods for Glipizide and Metformin Hydrochloride Tablets, found in USP30-NF25. The compendial content method for glipizide is a reversed phase method using an 8 minute isocratic profile, a C_{18} column with potassium phosphate buffer and acetonitrile as mobile phases at pH 6.0. The compendial content method for metformin is a UV absorbance method at 233 nm. Although both methods are acceptable for use for the analysis of those compounds, both methods had to be developed and validated. For every one Metaglip™ tablet analysis, two methods have to be used and two sets of data generated. As demonstrated in this paper, a single 5 minute cation exchange method could be used for the analysis of both components from a single preparation of Metaglip™. This translates to a considerable savings in time and resources for the developing lab.

In all modes of HPLC separation, consideration must be given to the mobile phase and diluent compositions to give optimal chromatography. Often drastic changes are needed when analyzing a compound using one mode of HPLC where using a different mode would only require subtle changes to achieve optimal chromatography. This paper provides a brief overview of reverse phase, HILIC, cation and anion exchange chromatographic techniques. While RP-HPLC might be the most common mode of analysis for small non-polar molecules, other chromatographic modes

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exist that are potentially superior to RP-HPLC, especially, when considering the time, complexity, and cost associated with development, validation, and routine analysis of combination pharmaceutical drug products and associated API. HILIC works well for retention of polar molecules; however, mobile phase composition may be tedious for mixtures of both acidic and basic molecules. Ion exchange chromatography can be successfully used to analyze combination drug products of varying polarity levels that yield robust, fast methods.

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Received December 12, 2008
Accepted March 19, 2009
Manuscript 6442

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