

## Lab Report

**Objective:** Utilize High Performance Liquid Chromatography (HPLC) to test the appropriateness of the NORM-JECT polypropylene/polyethylene syringes for use in sample preparation, transfer and filtration of samples prior to analysis on HPLC instrumentation.

**Bottom Line:** NORM-JECT syringes from 5 different batches and sources, including both sterile and non-sterile, were challenged with a wide variety of solvents and solutions typical for use with Reversed Phase and Normal Phase HPLC and were found to be suitable. In addition, a competitive 3-part syringe having a black rubber plunger tip was similarly tested and found to be not suitable for use with organic solvents of several types.

## Experimental

The NORM-JECT syringes are used in many ways in a typical analytical laboratory. In our lab, we use them to draw up viscous and semi-solid samples from their containers and vials. We also use them to weigh such samples prior to placing into volumetric glassware, as well as to withdraw samples which have been dissolved and diluted, then to push through micro-porous membrane syringe filters to remove particulates prior to HPLC analysis. Other labs we have visited in the course of our conducting HPLC training classes are using them in a similar fashion. In all these steps, which are part of sample preparation prior to HPLC analysis, the syringes are exposed to a variety of solvents, solutions and buffers for only a few minutes. It is critical however, that the syringes do not contribute foreign substances into the sample, thus contaminating the sample and producing false results. Such contamination would be seen as additional peaks in these chromatograms, in addition to those which actually come from the sample. In many cases these peaks might need to be reported as contaminating substances in the sample, the source of these peaks often not recognized as coming from the syringe, and could be cause for rejection of a product or might bias research results.

In order to test the NORM-JECT and 3-part syringes for appropriateness in HPLC analytical procedures, they were challenged with a variety of commonly used solvents and solutions which were then analyzed to see if any detectable substances were observed in resulting chromatograms. The solvents and solutions included in this study were: HPLC grade water, HPLC grade water containing 0.1% trifluoroacetic acid, 1N hydrochloric acid, HPLC grade water containing 0.1% formic acid, 1N sodium hydroxide, 10% acetic acid, methanol, acetonitrile, ethanol, isopropanol, tetrahydrofuran, dimethylsulfoxide, dimethylformamide, methylene chloride, chloroform, and hexane. In all, the syringes were challenged with 16 different solvents and solutions which are commonly used in preparing samples for HPLC analysis. Since these syringes normally are exposed to such materials for only a few minutes, it was believed that a 1 hour exposure to them would be more than sufficient to determine suitability. Much longer term exposure might well produce different results as well as damage and/or swelling of the polypropylene or polyethylene materials.

All syringes were supplied by the manufacturer Henke-Sass, Wolf GmbH through their US partner Air-Tite Products Co., Inc.. The specific syringes which were included in the testing protocol were as follows.

HSW Part Code 4010-200V0 (Air-Tite Part Code A1), 1mL NORM-JECT Luer Slip Syringe with green plunger, Sterile, Lot# 1K17048, Manufacturer: HSW

HSW Part Code 8300013982 (Air-Tite Part Code ABC3LS) 2mL (3mL) NORM-JECT Luer Slip Syringe with white plunger, Bulk Non-Sterile Convenience Pack, Lot# 0M06040, Manufacturer: HSW

HSW Part Code 4050-000VZ (Air-Tite Part Code A5), 5mL NORM-JECT Luer Slip Syringe with white plunger, Sterile, Lot# 0H23048, Manufacturer: HSW

HSW Part Code 4100-X00V0 (Air-Tite Part Code AL10), 10mL NORM-JECT Luer Lock Syringe with white plunger, Sterile, Lot# 1E04048, Manufacturer: HSW

HSW Part Code 480003000 (Air-Tite Part Code AL30), 30mL NORM-JECT Luer Lock Syringe with white plunger, Sterile, Lot# 09L048B, Manufacturer: HSW

Part: BD309604 10mL 3-part Luer Lock syringe with clear plunger having black rubber tip, Sterile, Lot# 0022508, Manufacturer: BD

### **Protocol**

The testing protocol followed was to fill each of the six syringes with sufficient volume of each of the test solutions and solvents. The 1mL and 3mL syringes were completely filled, the 5mL and 10mL syringes were half-filled, and the 30mL syringes were filled to the 10mL level. Each syringe was then exposed to its respective solution or solvent for a timed 1 hour period, the test sample was then shaken to mix thoroughly and placed into a glass auto-sampler vial, capped, then placed into the chromatograph for analysis. So as to avoid possible contamination from other materials, the syringes were not capped, nor were the samples filtered. There were thus 96 samples in all which were tested in this manner.

The chromatographic system used was a Waters Corp. Acquity UPLC instrument made up of a binary gradient pumping system, auto-sampler, and diode array UV absorbance detector. The first 13 solvents and solutions in the above list were chromatographed using a reversed phase system which included a C18 column, 2.1mm x 50mm, containing 1.7 $\mu$ m packing. The mobile phase A was HPLC grade water adjusted to pH 2.2 with HPLC grade phosphoric acid, and mobile phase B was HPLC grade acetonitrile. The final 3 solvents (Methylene Chloride, Chloroform and Hexane) were chromatographed using a normal phase system on the same instrument. In this case, a silica column, 4.6mm x 150mm, containing 7 $\mu$ m packing was used. Mobile phase A was HPLC grade hexane and Mobile phase B was isopropanol. In both systems a 0-100% gradient was used to chromatograph the samples and the UV detector was set at 220nm wavelength. The extensive gradient as well as the 220nm wavelength was chosen in an effort to discover any peaks which would likely result from the solvent challenge. In the end, however, all wavelengths from 210nm to 300nm were inspected in case substances absorbing at these wavelengths were extracted from the syringes. This wavelength range therefore, covered the vast majority of the UV spectrum which would likely be used by any typical HPLC method. Additionally, in the case of all test samples a blank injection of the fresh solvent or solution, used for the test, was also chromatographed to provide a means of comparison. Thus there were 16 chromatograms which were produced from these "solvent blank" samples. As is typical, these solvents and solutions did produce "system peaks" when they were chromatographed, so the chromatograms of those samples which were recovered from the syringes were inspected for the presence of any additional peaks which would arise from the syringes.

## **Results**

Once all 112 samples and blank injections were completed, an inspection of each chromatogram was made, including not only those produced at 220nm wavelength but also those at other wavelengths covering the range from 210nm to 300 nm as discussed above. All chromatograms resulting from the solutions and solvents taken from each syringe were overlaid using the operating computer program for comparison with the "solvent blank" chromatogram. (See attached examples included with this report). Any peaks in those chromatograms which were not also generated by the blank injections were considered to have been extracted from the syringes and thus would be unacceptable for HPLC use.

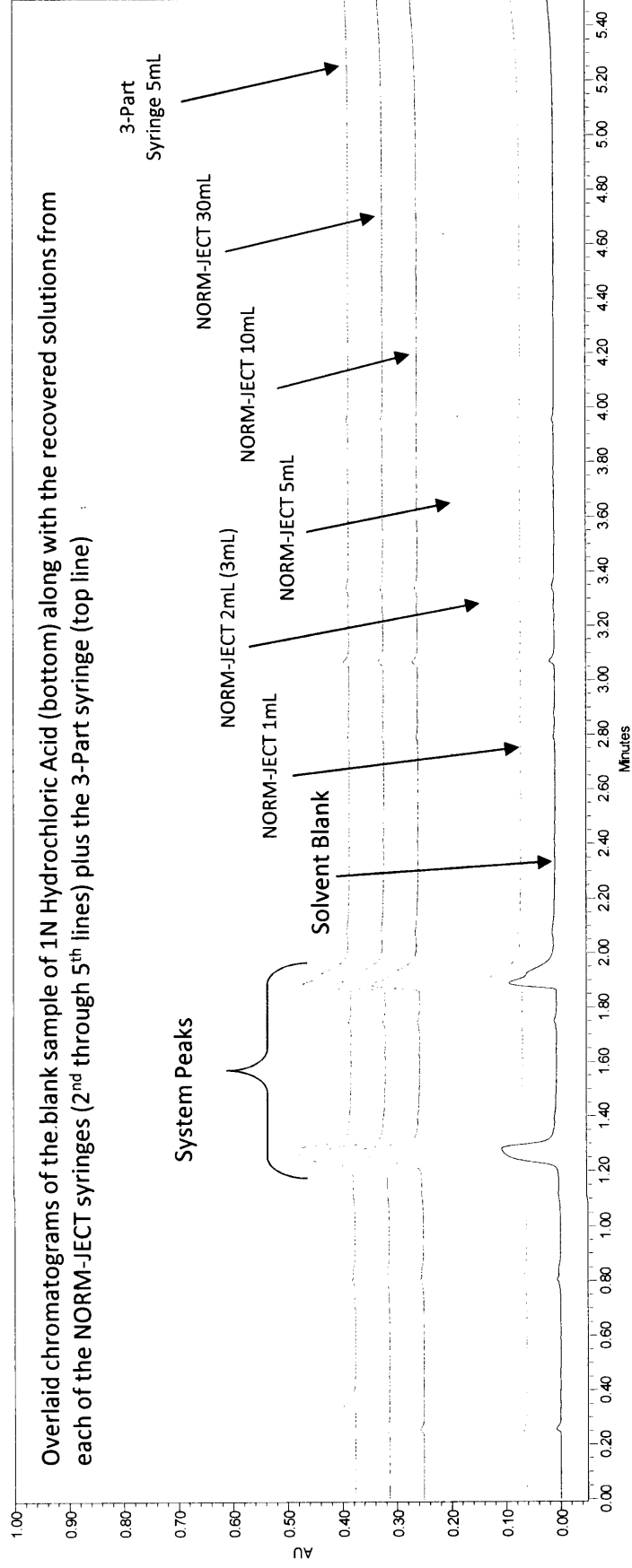
What was observed in our findings was that the six aqueous solutions (water, acid and base solutions) were compatible with all syringes including the 3-part type. No physical damage was observed and no contaminating peaks were found in the chromatograms. The organic solvents however, resulted in a different conclusion. All of them were compatible with the NORM-JECT syringes, but none of them were without problems for the 3-part syringes. The least problematic were the relatively polar alcohols, acetonitrile, dimethylsulfoxide, and dimethylformamide which produced several small peaks in the chromatograms. The worst were the intermediate polarity tetrahydrofuran and the non-polar methylene chloride, chloroform, and hexane. These produced one or more large peaks in the chromatograms and also began to physically damage the syringes by swelling the black plunger tip to the point that once removed from the syringe barrel, could not be re-inserted.

## **Final Conclusion**

The NORM-JECT syringes performed very well and showed no signs of physical damage nor did they produce contaminating peaks in any chromatograms when challenged for 1 hour by any of the commonly used solvents or solutions. We highly recommend these all plastic syringes for use in normal and reversed phase HPLC sample preparation procedures and expect them to perform well for other forms of chromatographic analysis techniques such as ion-exchange, hydrophobic interaction and size exclusion chromatography which use various aqueous salt and buffer solutions during sample preparation protocols. We therefore certify the NORM-JECT syringes for HPLC sample preparation use.

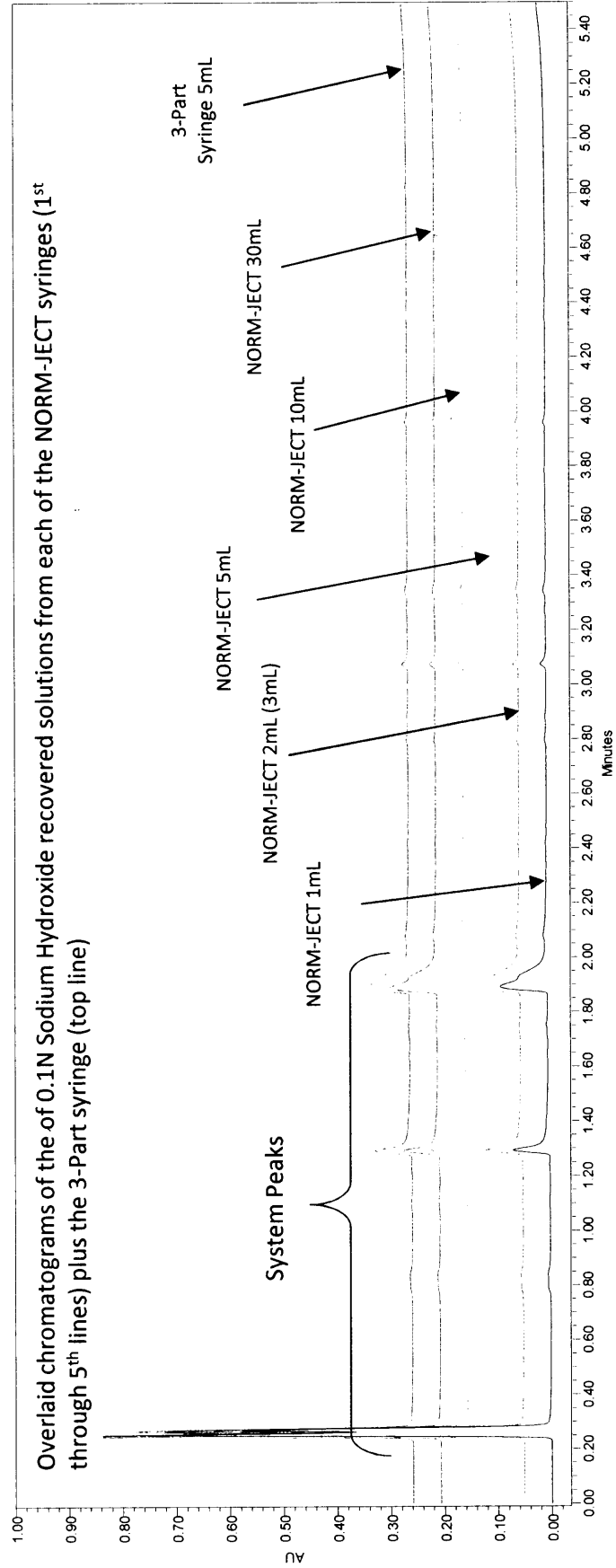
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# 1N HCL Test Solution



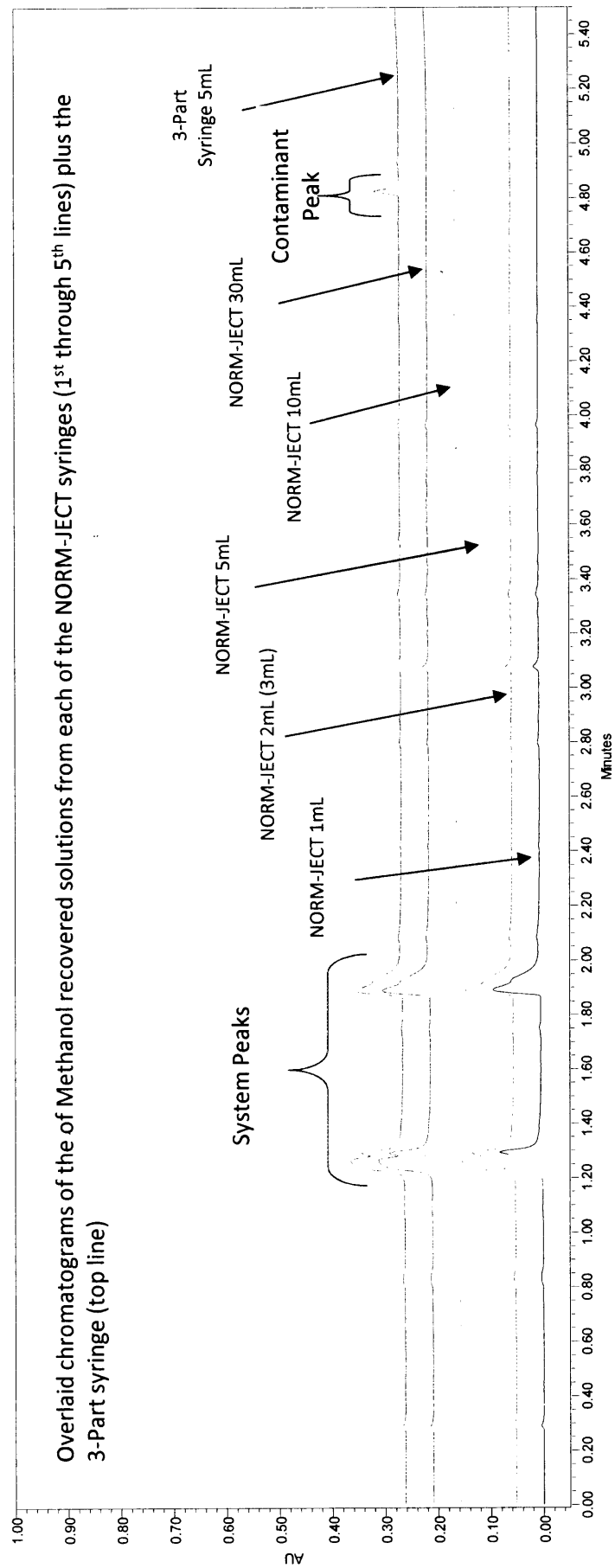
Conclusion: all chromatograms are essentially the same as the blank. No contaminating substances were extracted from any syringe.

# 0.1N NaOH Test Solution



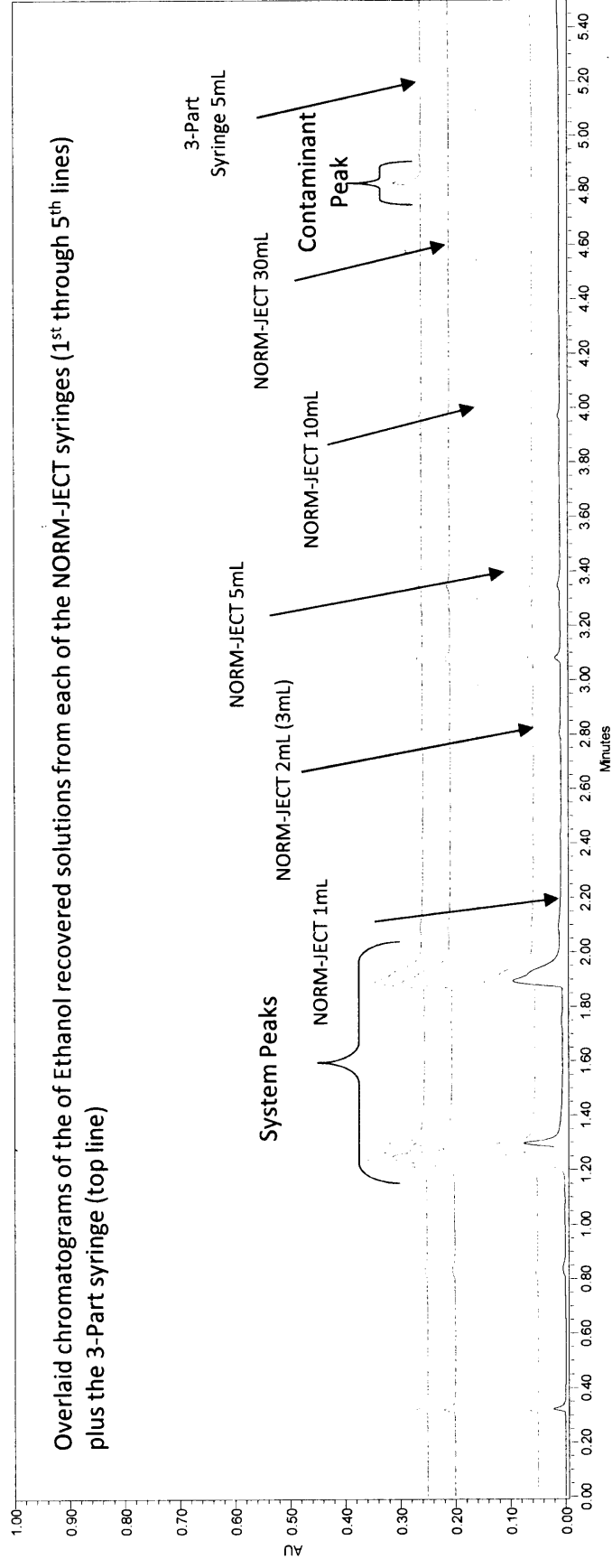
Conclusion: all chromatograms are essentially the same as the blank. No contaminating substances were extracted from any syringe.

# Methanol Test Solution



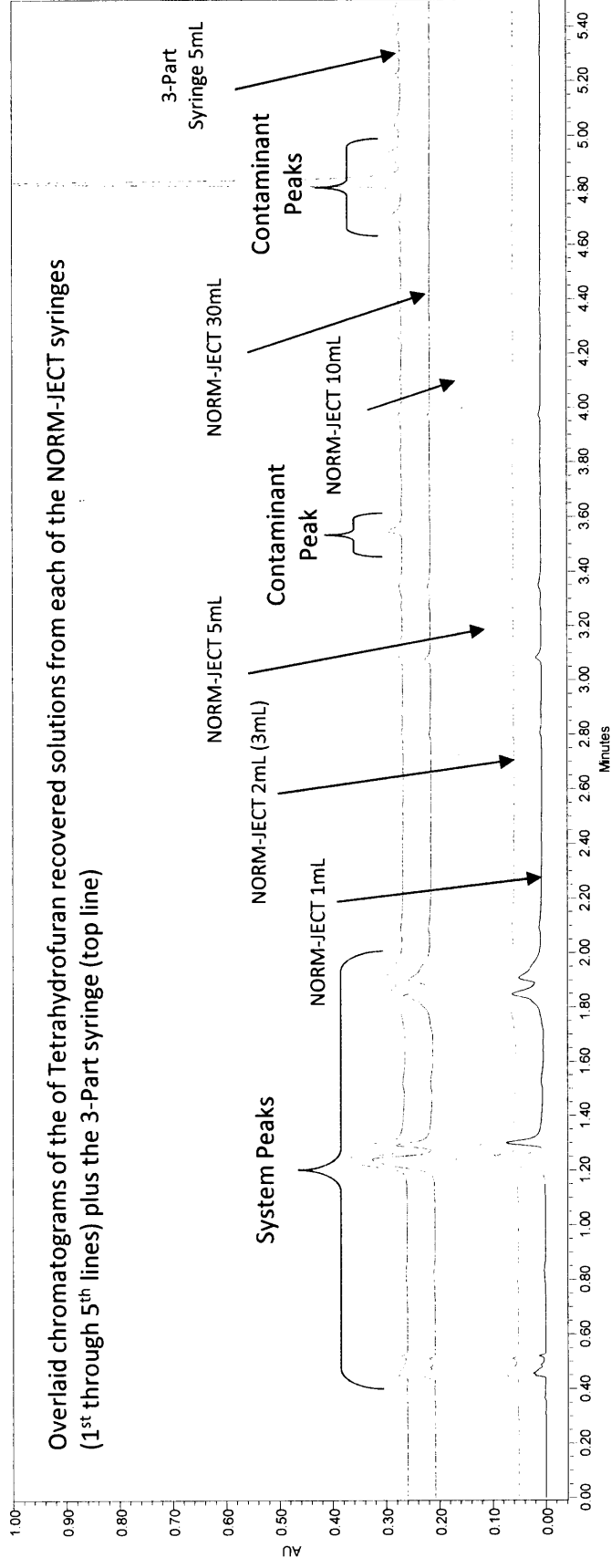
Conclusion: all NORM-JECT chromatograms are essentially the same as the blank. No contaminating substances were extracted from any NORM-JECT syringe. A contaminating substance was extracted from the 3-part syringe.

# Ethanol Test Solution



Conclusion: all NORM-JECT chromatograms are essentially the same as the blank. No contaminating substances were extracted from any NORM-JECT syringe. A contaminating substance was extracted from the 3-part syringe.

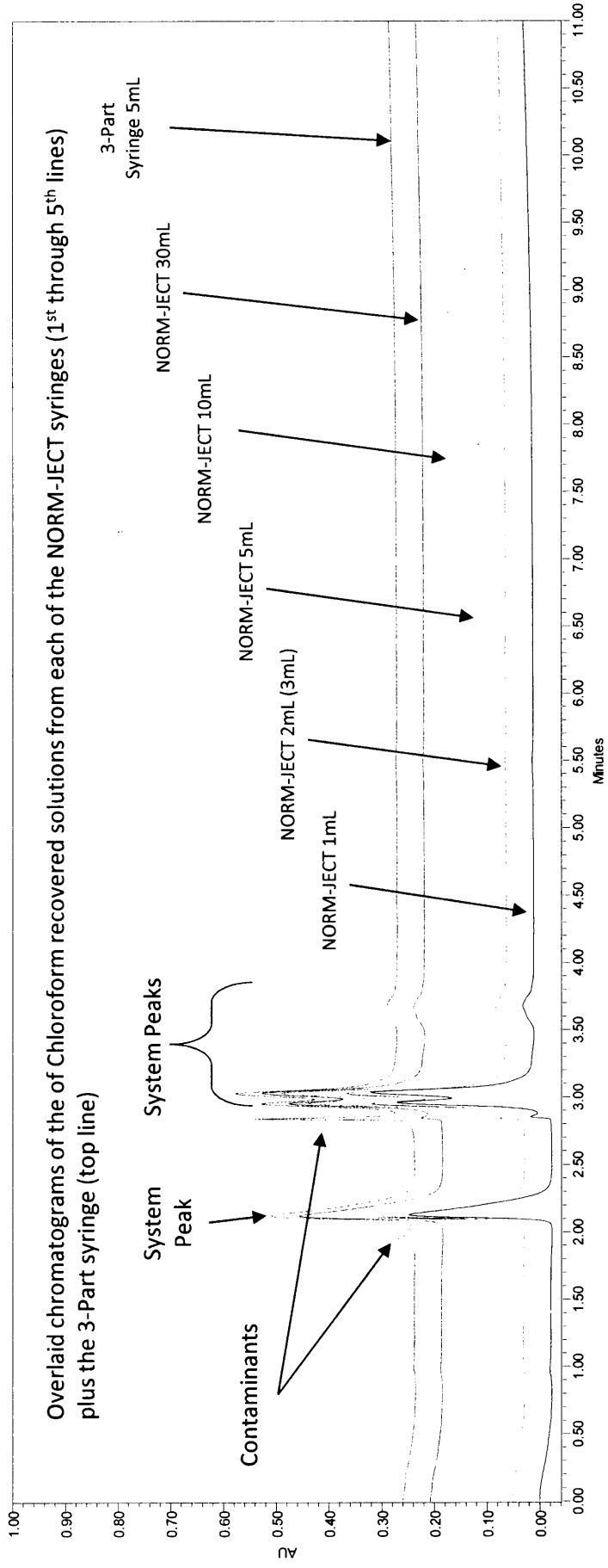
# Tetrahydrofuran Test Solution



Conclusion: all NORM-JECT chromatograms are essentially the same as the blank. No contaminating substances were extracted from any NORM-JECT syringe. Contaminating substances were extracted from the 3-part syringe.



# Chloroform Test Solution



Conclusion: all NORM-JECT chromatograms are essentially the same as the blank. No contaminating substances were extracted from any NORM-JECT syringe. Contaminating substances were extracted from the 3-part syringe.