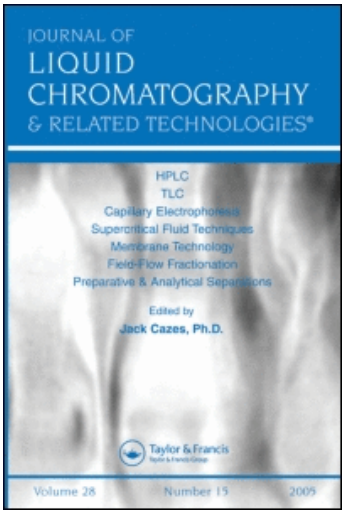

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A Universal HPLC Determination of Insulin Potency

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A UNIVERSAL HPLC DETERMINATION OF INSULIN POTENCY

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ABSTRACT

A liquid chromatographic method for determination of insulin potency is presented. It is applicable to bovine, porcine and human insulins, and to both insulin crystals and insulin formulations. The method uses a unique standard preparation (40 U/ml in 0.6 mg/ml EDTA) which is stable enough to permit use for >1 month. Likewise, samples are prepared in a stable form, so that an autoinjector may be used. NPH insulin suspensions are clarified by addition of heparin, lente insulins are clarified by addition of tetrasodium EDTA, and EDTA is added to neutral regular insulins and insulin crystals. Chromatography was done using a previously described 0.2 M pH 2.0 phosphate/26% CH_3CN mobile phase and a reversed phase column. A three-laboratory study of the method found it to have a precision of ±2% for formulation assays. It was verified to be accurate by comparison with the traditional rabbit biopotency assay, over which the liquid chromatographic assay has several significant advantages.

INTRODUCTION

HPLC is a very powerful tool for analysis of peptides and proteins. As this fact has been recognized and appropriate chromatographic conditions worked out, there has been an explosive growth in the use of HPLC for peptide and protein assays. The analysis of insulins has traditionally

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been done with biological assays (1). It is now widely recognized that appropriately developed chromatographic assays are superior to biological assays for many applications (2). Although there have been a large number of reports of studies of the reversed phase chromatography of insulins (3-6 and references therein), only one of the reports dealt with insulin formulations (4). The work described below arose from a need to quickly and efficiently identify and determine the potency of insulin crystals and insulin formulations. During the development of such an assay it became apparent that stable standard and sample preparations were needed. All previous papers, including the only formulation assay (4), dissolved the insulin in dilute acid. This is adequate for qualitative work such as the identification of insulins from different species. However, at room temperature and low pH insulin undergoes deamidation reactions, principally at the A-21 asparagine. It is inconvenient to acidify samples immediately prior to injection and thus an alternative was sought. The resulting assay, which provides for stable standard and sample preparations, and is applicable to human and pancreatic insulins in both crystals and formulations, is described below.

MATERIALS AND METHODS

Reagents

HPLC grade acetonitrile was purchased from Burdick and Jackson. The 85% phosphoric acid, tetrasodium ethylenediaminetetraacetic acid (Na_4EDTA), and the monobasic sodium phosphate (Na_2HPO_4) were of analytical reagent grade. The sodium heparin purified was purchased from Diosynth, Inc. Purified water was produced using a Milli-Q purifying system purchased from Millipore Corp. Crystalline human,

pork, and beef insulins and insulin formulations were prepared at Eli Lilly and Company.

Apparatus

Several HPLC systems were used during the development of this method. They consisted of either a variable wavelength detector operated at 214 nm (such as a DuPont spectrophotometer) or a 214 nm single wavelength detector (such as a Waters Model 441), an automatic sampler, a column oven or block heater, and a suitable pump. Several pumps were used, including the Waters Associates Models M-45 and M-6000A, the Altex Model 110A, and the Beckman Model 112. Data collection and reduction was done on a central computer which provided extensive data calculation and graphics capabilities. The HPLC columns used in this study were the DuPont Zorbax TMS and the Brownlee Aquapore RP-300. Both columns were analytical columns of 25 cm x 4.6 mm dimensions. Most of the work reported in this paper was done using DuPont TMS columns.

After filtration, the column eluting solution was maintained in a closed system consisting of a reservoir for the main solution and a smaller secondary reservoir which contained acetonitrile. These two reservoirs were connected to each other by a length of Teflon tubing whose ends were above the two solutions. Other than this tube and the inlet line for the HPLC pump, these reservoirs were sealed. The main reservoir was continually stirred at low speed using a magnetic stirrer. The second reservoir maintains the vapor pressure of acetonitrile in the main reservoir, in order to prevent evaporative losses of acetonitrile from the mobile phase. This allowed large volumes of mobile phase to be prepared and used without changes in the retention time of insulin.

A DuPont model 950 thermogravimetric analyzer (TGA) was used to determine the volatiles content of 10 mg samples of the insulin crystals and insulin reference standards. A heating rate of 5°C per minute and nitrogen atmosphere (at a flow rate of 40 ml/min.) were used.

Chromatography

The mobile phase was prepared by making a solution of 0.1 M NaH_2PO_4 and adjusting the pH to 2.0 by addition of concentrated phosphoric acid. This solution is then mixed with acetonitrile in the proportions 74:26 (buffer:organic). The mixture is filtered and degassed under vacuum. The flow rate was normally 1.0 ml/min. If necessary the mobile phase and/or flow rate were adjusted slightly to achieve a retention time for human insulin of about 1000 to 1300 seconds. All samples were injected at room temperature. The injection volume was 10 or 20 microliters. The column temperature was 40°C.

System suitability criteria based upon the separation of insulin and its A-21 desamido derivative were used to ensure the quality of HPLC separations run at any given time and place. A solution of human insulin is prepared at a concentration of about 1.6 mg/ml. A portion of this solution is chromatographed. The resolution factor (R) between the insulin and the desamido insulin peaks is to be not less than 1.8, and the tailing factor (T) of the insulin peaks is not less than 1.8.

$R=2(t_2-t_1)/(W_2+W_1)$, where t_2 and t_1 are the retention times of the desamido and the insulin peaks respectively, and W_2 and W_1 are their peak widths measured at 5% of peak height. $T=W_{0.05}/2F$; where $W_{0.05}$=width of insulin peak at 5% of its peak height and F=the width from the leading edge of the peak at 5% of the peak height to the peak maximum.

Preparation of Insulin Standards

Standard curves were made by accurately weighing quantities of the appropriate species insulin reference standard to give concentrations of about 34, 40, and 45 USP insulin units per ml at a pH of between 8.5 and 9.0. This was achieved by dissolving the standard in a solution of 0.6 mg/ml Na_4EDTA to which 6 ml of 0.1 N HCl had been added per liter. Complete dissolution of the solids may require several hours. The solutions may be sonicated briefly (≤15 seconds) to expedite this process. A portion of each standard was injected into the chromatographic system. The chromatograms were recorded and area responses for the main insulin peak determined. A standard curve was constructed by plotting the standard concentration versus the measured peak areas, using the linear least squares method. The curve was not used if the relative standard deviation of the points from the line was more than 1.6%. These standard solutions were kept refrigerated and used for up to one month before being discarded.

The desamido content of the samples was quantitated by comparison of its peak area to that of a standard diluted as follows. One ml of approximately 40 U/ml standard solution was transferred into a 50 ml volumetric flask and diluted with 0.6 mg/ml Na_4EDTA solutions. The quantity of desamido in USP Insulin units was multipled by 0.9, which is the biological activity of A-21 desamido insulin relative to that of insulin. Total potency was determined as I+0.9d; where I=potency of insulin and d=potency of desamido as calculated from an insulin standard.

Preparation of Samples

The potency of insulin crystals was determined by quantitative preparation of an approximately 40 U/ml solution of the sample in the

0.6 mg/ml EDTA solution used for preparation of standards. Three replicate weighings and dilutions were always made. If the volatile-free potency was to be determined, then a TGA determination of volatiles in the sample was obtained concurrently.

Insulin formulations were prepared for chromatography by the following procedures. For neutral regular insulins of 40 U/ml concentration, 0.6 mg of tetrasodium EDTA per ml of formulation was weighed into the vial, or a small volume of a concentrated solution of tetrasodium EDTA was added to the vial. Neutral regular insulins (NRIs) of greater concentration were diluted with a Na_4EDTA solution to yield a final insulin concentration of about 40 U/ml and a Na_4EDTA concentration of 0.6 mg/ml. Protamine insulin suspensions (NPHs) were first clarified by addition of an 80 mg/ml solution of sodium heparin in water. This reagent was stored refrigerated, but both samples and heparin solution were allowed to come to room temperature prior to mixing. One microliter of the heparin solution was added for each 20 Units of insulin present. After the suspension clarified the sample was treated with tetrasodium EDTA and diluted if necessary to obtain a 40 U/ml solution containing 0.6 mg EDTA/ml. Lente (zinc insulin) suspension formulations were clarified by addition to the vials of 1 mg of tetrasodium EDTA for each 16 Units of insulin. If necessary the solutions were then diluted to achieve a 40 U/ml insulin concentration. Three injections and/or replicate dilutions of each formulation were made in all cases. If the relative standard deviation of replicate preparations of a single sample was greater than 1.6% the assay was repeated.

Method Validation

The reproducibility of the HPLC potency assay was tested by comparing the results obtained by three laboratories for samples from

TABLE 1

Average Assay Results for NRI,NPH Assay Validation Study

Lot	Type	Label (units/ml)	Lab average (% label) 1	2	3	Average n
1	NRI	100	104.2	104.1	105.9	9
2	NRI	100	99.0	99.9	100.1	9
3	NRI	100	100.1	102.2	101.9	5
4	NRI	80	99.1	100.5	99.4	3
5	NRI	40	101.5	103.3	101.0	9
6	NPH	100	103.9	105.4	105.7	9
7	NPH	80	96.8	96.5	97.8	3
8	NPH	40	101.0	102.3	101.3	9
9	NPH	40	101.5	103.3	101.0	9

the same lots. Nine lots were used, including NRI and NPH formulations in 40, 80, and 100 unit/ml concentrations. Most of the lots were assayed over the course of several days in each lab, generally using 3 replicate assays per day on 3 days. This study design allowed the assessment of variation within each lab as well as that from one laboratory to another. A summary of the lots used and number of assays run in each laboratory is shown in Table 1.

An additional limited study was carried out for the Lente formulation. One lot of Lente insulin was assayed by 2 analysts from one of the labs using separate instruments. Ten replicate assays were obtained on a single day. Tnis smaller study does not permit the assessment of lab-to-lab variation but does still provide some useful information with respect to the variability of the assay for Lente formulations.

One source of variation which was not addressed by either of the studies described above was that due to variability in the standards, either through variation in preparation techniques or volatiles corrections, since each study used a common standard solution throughout. A separate data set was thus needed in order to measure any

variability induced by the standards. Nine samples were assayed and their results were calculated relative to the average of two 3 point standard curves for each of 4 separately prepared sets of standards. This data was used to estimate the variation in the results which was induced by variability in the standard curves.

RESULTS AND DISCUSSION

Chromatographic Conditions

The specific chromatography conditions used in this work were previously described by Kroeff and Chance (5). They readily separated bovine, human and porcine insulins, and A-21 desamidos from intact insulins. Typical chromatograms are depicted in Figures 1 and 2. Low pH phosphate buffers have been widely used for the reverse phase chromatography of proteins and peptides, most frequently in combination with acetonitrile as the organic modifier. The use of a low pH mobile phase with reasonable ionic strength frequently provides for good peak shapes. Phosphate buffers are of particular interest because of their good buffering capacity around pH 2.1 and their very low absorbance in the range of 210 to 220 nm where the greatest sensitivity of detection is realized for peptides. Typical column life during continuous use was about 4 to 6 weeks. Use of acetonitrile also commonly results in good peak shapes. As has been noted previously (7), the retention of insulin is quite sensitive to the percent acetonitrile in the mobile phase. Thus a 1% increase over the 26% specified here can cut the retention time of insulin in half and a 1% decrease can nearly double the retention time. In order to prevent overly frequent "titrations" of the mobile phase to achieve the desired retention time for insulin (about 20 minutes), relatively large batches of mobile phase (3 to 10 liters) were prepared at one time. To maintain retention times constant

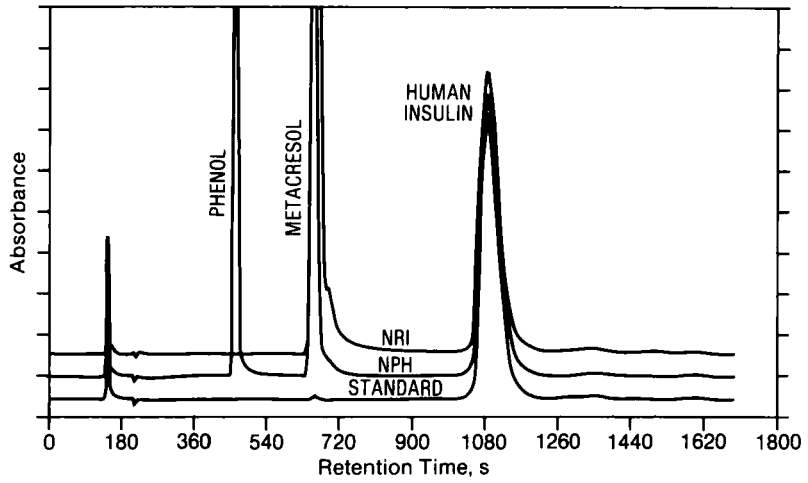

FIGURE 1. Representative chromatograms (on a TMS column) of a 40 U/ml human insulin standard, an NPH formulation and a neutral regular formulation. Conditions as per text.

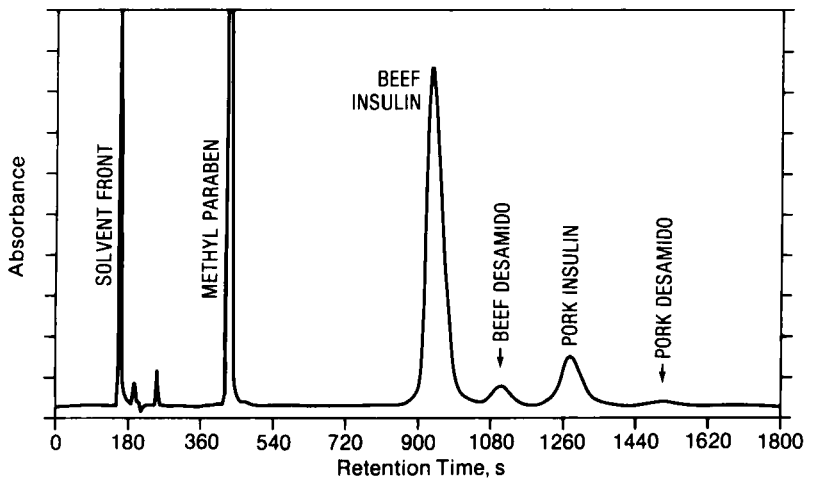

FIGURE 2. Representative chromatogram of a mixed beef/pork Lente formulation. Conditions as per text.

over several days time, the mobile phases were placed in the reservoirs described above, which served to prevent evaporative losses of acetonitrile.

As has been noted previously, operation of the chromatographic column at elevated temperatures enhances the retention of insulin and column performance. Maintenance of constant column temperature of course improves the precision of retention times and assays.

This assay was primarily developed using the Zorbax TMS column. It has also been extensively run on the Aquapore RP-300. Each column has its own benefits in terms of selectivity differences among various minor insulin-related substances. However, both are useful for this assay in that they resolve A-21 desamido insulin from insulin and they resolve beef, human and pork insulins. Recently, we have evaluated a series of columns for peptide and protein separation, including insulin. In terms of peak shape and efficiency, the Zorbax C8 150Å pore column was found to be preferable to all others tested. Work done in other laboratories with additional columns suggests that they might be suitable also (4,6).

Design of Standard and Sample Preparations

The design of this method was based upon the aforementioned need to avoid acidifying all samples and standards. It was decided to dissolve the insulins at neutral or slightly alkaline pH to reduce or eliminate deamidation. A uniform procedure for the treatment of the various forms of insulin was also sought. The use of ethylenediaminetetraacetic acid (EDTA) was found to be the key to designing such a method. Injection of 10 µl of neutral insulin solutions of 40 U/ml concentration in water were found to give spuriously high peak areas on some LC instruments. The addition of tetrasodium EDTA and/or raising the pH to

8.5 or higher eliminated this problem (a full report of the mechanisms of this effect will be attempted at a later date). A tetrasodium EDTA concentration of 0.6 mg/ml for a 40 U/ml standard solution was arbitrarily chosen as sufficient to complex all of the zinc present in the insulin crystals and to raise the pH of the solution.

The stability of standard solutions of human insulin in 0.6 mg/ml EDTA was studied as a function of pH and temperature. Portions of a 40 U/ml standard solution were adjusted to various pHs over the range 7 to 10, and were held refrigerated and at room temperature. Aliquots were assayed versus freshly prepared standards over a period of 144 hours. It was found that too high a pH was detrimental to the room temperature stability of insulin. The decrease in main peak insulin area as a function of time at pH 9.9 and room temperature is depicted in Figure 3. As the insulin peak area decreased, about 4 earlier and 2 later eluting components were observed to grow. The amount of insulin remaining after 144 hours at room temperature is depicted as a function of pH in Figure 4. The loss of insulin clearly accelerates above pH 9. No decomposition could be detected in the refrigerated samples; the average ratio of aged to fresh samples was 0.996. Still, in order to make the method as rugged as possible, a pH below 9 was chosen for the standards, to maximize the time which they could be left on an autoinjector prior to chromatography. In addition, it was desired that relatively large amounts of standards be prepared at one time, held refrigerated and used for several days at least. To verify the feasibility of this approach, longer term stability data on refrigerated standard solutions was obtained. For 6 human insulin standard solutions with pHs above 9.0, a least squares linear fit to the potency as a function of time indicated decomposition rates of from 0.0 to 0.5% per month in the refrigerator. With standard solutions of pH 8.5 to 9.0, no

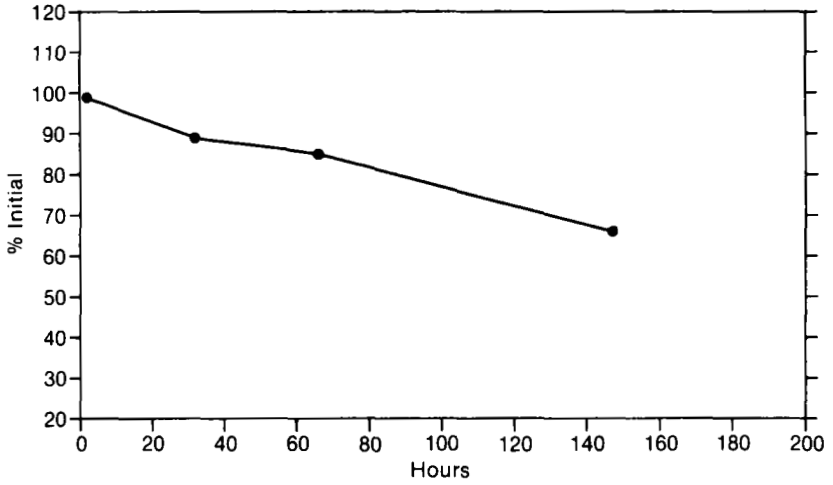

FIGURE 3. Decomposition of human insulin standards in EDTA solution at room temperature and pH 9.9.

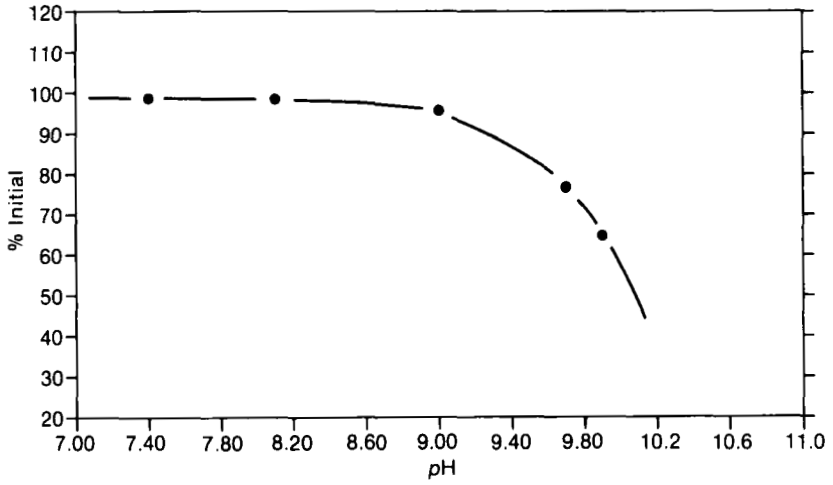

FIGURE 4. Human insulin standard curve stability. Conditions: 144 hours at room temperature in 0.6 mg/ml EDTA.

degradation could be detected after 6 months. This confirmed the importance of pH adjustment of the standards for maximum stability. With this procedure, standard curves can be prepared monthly or even less frequently. Our laboratories assay a large number of insulin samples, and less frequent preparation of standards increases efficiency. In addition, since larger weights and volumes are used, greater precision is realized. Quality control is also improved, since each new standard curve can be validated by comparison with the previous curve.

The design of the method with regard to preparation of samples for chromatography was to provide stable solutions of approximately 40 U/ml concentration. Thus all types and strengths of insulin samples (of a given species origin) can be assayed versus a single set of standards. This allows for convenient and efficient assay of many samples. An additional benefit is the quality control mentioned above, which would not be realizable with multiple sets of standards to be kept track of.

Bulk insulin crystals can of course be handled the same as standards. Neutral regular insulin formulations are stable and completely solubilized, so it was only necessary to add EDTA to them and to dilute to 40 U/ml if necessary. As with the standards, it was found that EDTA should be added to these formulations to eliminate any possible matrix effects.

The isophane insulin suspension (NPH) formulations presented a special problem. These are readily solubilized with acid, but the resulting solution is not stable. It was found that the protamine-insulin complex could not be broken with tetrasodium EDTA. However, protamines are commonly used as heparin antagonists, and indeed it was

found that the protamine and insulin could be solubilized by addition of heparin sodium. A study comparing the addition of various amounts of heparin showed the specified amount to be sufficient for all NPH's tested. If an excess of heparin is added, very poor peaks for insulin can result. It was also found that the reaction must be run at room temperature. If the NPH sample and/or the heparin solution are cold, the formulation may not clarify upon addition of the heparin. If this occurs, warming the solution and/or addition of more heparin will not clarify the sample. After clarification of NPH samples, EDTA was added for the sake of consistency with standards and other samples. As a result of the buffering capacity of the protamine, the addition of the tetrasodium EDTA does not raise the pH of the sample as it does with neutral regular and zinc insulin suspension formulations.

A third type of formulation which was studied was Lente suspensions. These are suspensions consisting of a mixture of amorphous and crystalline zinc insulins. Extra zinc is added in the preparation of this formulation, above that originating from the bulk insulin crystals. It was found that tetrasodium EDTA would readily clarify these suspensions, but amounts greater than the 0.6 mg/ml used with standards and the other formulations were required.

Method Characteristics

The linearity of the chromatographic assay was assessed by preparing five insulin solutions over the range 0.94 to 2.06 mg/ml. This range brackets the working range specified above. Chromatography of these solutions showed the method linearity to be excellent: a correlation coefficient of 0.9998 was obtained and a maximum deviation from the least squares fit line of 0.56% was observed.

The accuracy of the method was evaluated by matrix experiments. In these experiments, a single pipet was used to transfer a constant amount of a single concentrated insulin solution to a series of volumetric flasks. These were then diluted to volume with appropriate dilution and treatment solvents to obtain simulated standards, insulin in mobile phase, and simulated "treated" formulations. Since the final insulin concentrations were identical, and a fixed loop injector was employed, identical insulin peak areas should result. Within experimental error, this was found to be the case. In the case of Lente insulins, the formulation is too complex to properly simulate, so EDTA treatment was compared to clarification of the same samples with acid.

The mean data from the interlab validation study using NRI and NPH material are summarized in Table 1. To estimate the precision of the HPLC assay, the data set was analyzed using a variance components estimation technique, accounting for the variability in assay results between as well as within laboratories. Although there was some variation from one laboratory to another, the major portion of the variability occurred among results obtained on the same day in the same laboratory. An estimated relative standard deviation (rsd) was also obtained for the Lente data. Since the validation study for Lente insulin was based on only one laboratory, the Lente rsd only pertains to the variability among results within a laboratory. The variation introduced by the standards, estimated to be 1.6% in the case where results are calculated against the average of two 3 point curves, was also included in the estimated overall relative standard deviation. The estimated overall rsd's for assay results within a given lab are shown in Table 2. Overall variation of the assay including variability from one lab to another is shown in Table 3.

TABLE 2

Estimated Relative Standard Deviations for HPLC Assay Results Within a Laboratory

Formulation	Number of replicates: 1	2	3
NRI	1.9%	1.8%	1.7%
NPH	2.3%	2.0%	1.9%
Lente	1.8%	1.7%	1.7%

TABLE 3

Estimated Overall Relative Standard Deviations for HPLC Assay Results

Formulation	Number of replicates: 1	2	3
NRI	2.1%	2.0%	1.9%
NPH	2.4%	2.2%	2.1%

The room temperature stability of samples prepared for chromatography was assessed by preparing 11 samples of various human insulin formulations and storing a portion refrigerated while the remainder was left for 3 days at room temperature. Relative to the refrigerated samples, six NPHs showed a decrease after 3 days of from 0.5 to 1.8%. Three neutral regular formulations decreased from 1.2 to 1.9%, and two Lentes lost 4.2 and 4.5%. Based on these results it was concluded that NPH and neutral regular formulations could be left on a turntable overnight without significant losses. The large decrease in the Lentes reflects the high pH which results from addition of the excess tetrasodium EDTA. In order to better characterize Lente sample stability, additional experiments were carried out.

One lot of each of seven insulin zinc suspensions (IZS) were used to further examine the stability of IZS sample preparations. One vial from each lot was clarified as described in the Methods section, and

TABLE 4

Stability of IZS Sample Preparations

	% Change from Initial After 48 Hours	
	Refirgerated	Room Temperature
pork Iletin™ II Lente™	-0.6	-3.2
Humulin™ all crystalline IZS	-1.0	-3.8
Humulin™ 70% crystalline IZS	-0.2	-1.7
Iletin™ I Ultralente™	-0.4	-5.2
Iletin™ I Lente™	-2.2	-6.5
Iletin™ I Semilente™	-2.8	-7.5
beef Iletin™ II Lente™	-2.0	-7.1

TABLE 5

Comparison of HPLC Potency and Rabbit Bioassay Results for Human Insulin Zinc Crystals

	$\bar{X}$	RSD	Range
Biopotency (U/mg)	28.06	2.8%	26.4-29.1
HPLC Potency (U/mg)	28.33	1.0%	27.8-29.0

from each vial, two replicate dilutions were made. One replicate was stored at room temperature, and the other replicate was stored in the refrigerator. The concentrations of the two replicates was determined initially, after one day and after two days of storage at the two different temperatures. The percent change after 48 hours was calculated based on a least squares fit of the data. The results are summarized in Table 4. After 48 hours, the pork formulation decreased by 0.6% at refrigerated temperature and by 3.2% at room temperature. The Humulin™ formulations decreased by an average of 0.6% at refrigerated temperature and by an average of 2.8% at room temperature. However, after 48 hours, the beef and the mixed beef-pork formulations decrease by an average of 1.9% at refrigerated temperature and by an average of 6.6% at room

temperature. This observation indicates that insulin zinc suspensions containing primarily bovine insulin are less stable than those formulated with either pork or human insulin. This difference in sample stability was not correlated with the pH of the sample preparation; all were between 9.32 and 9.65. Comparing the two Humulin™ formulations or the Ultralente, Lente and Semilente, there does not appear to be any correlation with the crystalline/amorphous ratio in the insulin zinc suspensions either. Based on the "worst case" degradation of beef IZS, prepared samples should remain at room temperature for less than 8 hours after preparation and at refrigerated temperature for less than 24 hours after preparation to insure that degradation remains below 1%.

Comparisons with other Insulin Assays

To validate the method described here, it was extensively compared to three other assays. Initially it was compared to the method which had been developed using hydrochloric acid solutions for dissolving and diluting standards and samples (5). For assay of insulin crystals, the current and the acid treatment methods were in excellent agreement. For neutral regular and NPH insulins, results on the average were slightly lower with the new procedure (1 to 3%) than with the old. Matrix effect experiments involving preparation of simulated standards and samples out of a single concentrated insulin stock solution indicated that the new procedure is the more accurate of the two.

The HPLC determination of insulin potency was also validated by comparison to the rabbit bioassay (8), run with analytical reference standard human insulin. The results obtained for 21 lots of human insulin zinc crystals are summarized in Table 5. A t-test based on the paired differences between the biopotency and the HPLC potency results

for each of the 21 lots showed no statistically significant difference in average results between these two assays (t=-1.56, p-value=0.13). Of course, the HPLC assay is significantly more precise ($p<.0001$) than the rabbit bioassay.

A third validation of the HPLC potency assay is the comparison with the results of a determination of insulin-related substances by HPLC (5). HPLC potency and HPLC purity results for 57 lots of biosynthetic human insulin were compared. The potencies for these lots ranged from 27.9 to 29.0 units/mg, while the purities ranged from 96.9% to 99.1%. The correlation coefficient for the potency versus purity results was 0.54, which is significantly different from 0 at the .0001 significance level.

Alternative Procedure for 100 U/ml Formulations

If only 100 U/ml formulations are to be assayed, standards may be prepared in 0.6 mg/ml EDTA around the 100 U/ml concentration. Complete dissolution of the standard insulin crystals at this concentration may take several hours. Injection volume should be limited to 10 µl. Samples of neutral regular insulin may be injected directly, NPH samples injected after clarification with 50 µl heparin solution (per 10 ml vial), and Lente samples injected after clarification with 50 mg of tetrasodium EDTA. The matrix effects observed in the absence of added EDTA at the 40 U/ml concentration were not seen at 100 U/ml, and so EDTA need not be added to regular and NPH insulins.

Simultaneous Determination of Preservatives in Formulations

Phenol and m-cresol, preservatives commonly used to prevent microbial growth in insulin formulations, can be determined simultaneously with

insulin. This approach conserves sample and offers greater efficiency in the routine analysis of insulin samples. The reagents required are the same as those given in the Methods section. The apparatus is also the same except that either a dual wavelength detector of two single wavelength detectors connected in series are needed. Non-linearity was observed at 214 nm, so detection of phenol and m-cresol is performed at 245 nm where the extinction coefficients for the two are lower. Sample preparation is the same as described in the Methods section. Evaluation of the linearity, precision and recovery of this approach as applied to preservatives in NPH, NRI and IZS formulations, found it to be good. The relative standard deviations were 1%, the log-log slopes were 1.02, and the recoveries 100 to 101%.

CONCLUSION

Reversed phase HPLC has been shown to be a precise and accurate analytical tool for the determination of the potency of insulins, both insulin crystals and formulations. As a result of the speed, precision, accuracy and specificity of this or improved chromatographic methods, it is to be expected that the biological tests of insulin potency will be replaced by HPLC methods.

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