the same principles. The instrument is easy to use once operators become familiar with its operation. Although the instrument was then evaluated for a few weeks no faults occurred which would raise doubts about its reliability in everyday use.

ACKNOWLEDGEMENTS
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Computer evaluation of the EMIT assays carbamazepine, ethosuximide, phenobarbital, phenytoin, quinidine and theophylline on the Gemsaec centrifugal fast analyser

Bengt Kinberger and Bengt-Åke Johansson
Department of Clinical Chemistry, Central Hospital, 301 85 Halmstad, Sweden

Introduction

The adaptation of EMIT antiepileptic drug assays to the Aminco Roto Chem II centrigufal fast analyser has been described by Finley et al [1,2]. The analysis procedure was very convenient and accurate; however, most of the data handling had to be done with the aid of a HP 9815A desk-top computer. Using Finley's modification of the EMIT assays the authors wished to adapt EMIT similarly to the Gemsaec centrifugal fast analyser and perform all the data processing with the Gemsaec computer. As no EMIT-program was available it was decided to develop one that would operate for routine clinical chemistry work. The program was written in FOCAL 8 (the version used by Electro Nucleonics Inc., the manufacturer of the Gemsaec) computer language. The Gemsaec transfer disc has only 16 positions, so it was considered important not to occupy a large part of the disc with standards when analysing unknown samples.

Apparatus

A Gemsaec centrifugal fast analyser attached to a PDP 8/e computer, with magnetic tape (dectape) as the storage device, was used for the evaluation. The Rotor temperature was kept within 37.0 ± 0.1°C. Electro Nucleonics Inc.'s loader for the Gemini analyser was used for the preparation of transfer discs for Gemsaec. The loader was prepared for the automatic delivering of two reagents and the sample flushed with buffer solution into the transfer disc.

Reagents

Reagent kits from the Syva Corporation were used through out the evaluation.

Stock solutions: Reagents A, B, aed-buffer and calibration standards were reconstituted according to Syva's recommendations.

Working solutions A and B : Stock solutions A and B were diluted 10-fold with aed-buffer solution according to Finley et al (2).

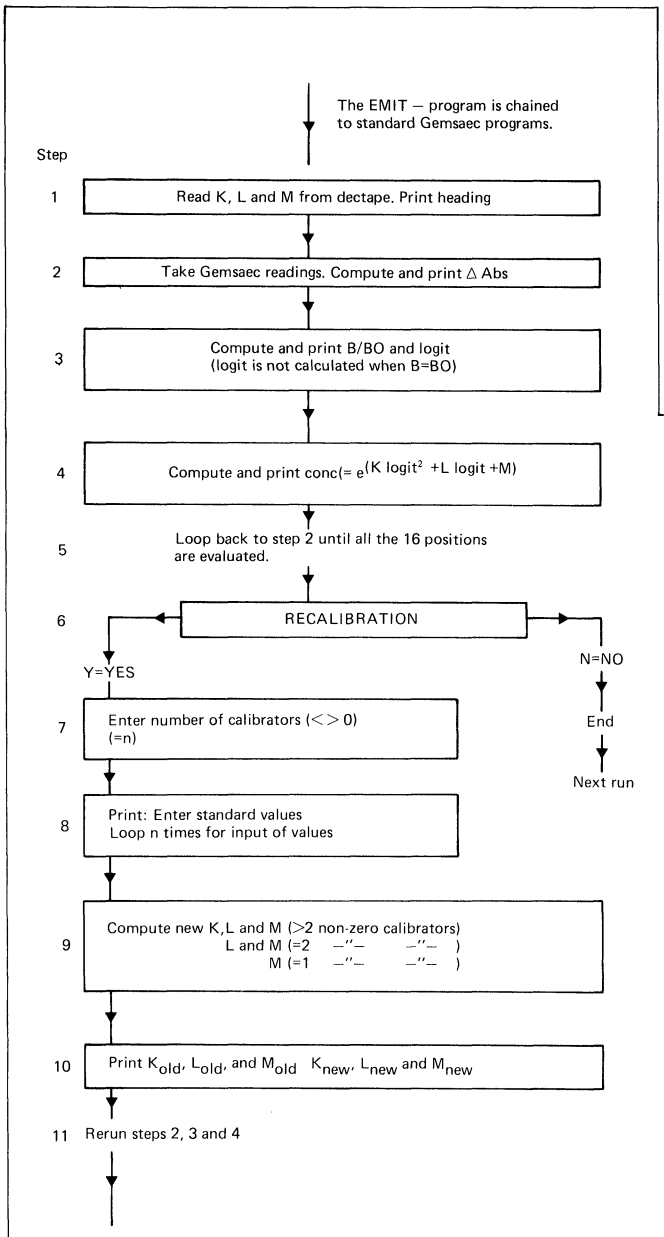
Flush solution : aed-buffer solution.

Instrumental parameters

Gemsaec instructions :
Reaction temperature : 37.0 ± 0.1°C
Wavelength : 340 nm
Filter position : 335-385 nm
Reaction mode : RATE
Initial reading : 45s
Reading interval : 60s
Number of readings : 5

Computer instructions :

IR	= 45	TC	= 17 ("address" to the EMIT program)
RI	= 60	CD	= 1
NR	= 5	AD	= 4
TF	= 70	SA	= 1.2



Transfer disc preparation

The transfer disc was prepared by means of totally automatic pipetting. 200 µl of working solution A was pipetted into well C of the disc. Simultaneously 200 µl of working solution B was pipetted into well B. 10 µl of the sample was flushed with 200 µl of aed-buffer solution into well C. The "sample holder" was loaded as follows: position 1 = deionised water, position 2 = zero calibration standard (blank), positions 3-7 = non-zero calibration standards (full set of standards) and positions 8-16 = unknown samples.

Data handling

Five absorbance readings were taken on each of the rotor positions. The delta absorbance, i.e. the last reading minus the first reading, is a measure of the enzyme activity of the sample in its cuvet. The enzyme activity is correlated to the concentration of the drug in the sample. Similarly to Finley et al [1] the measured delta absorbances were treated as free counts of radioactivity in the radioimmunoassay. Applying the logit-log transformation used by Finley et al, in the computation of the standard curve, a second order, unweighted, non-linear curve provided a better fit for the experimental data than a linear curve did.

The following formulas were used as the basis of calculation of the FOCAL program developed here:

(1) $B = 1 - \Delta Abs$

$B0 = 1 - \Delta Abs_0$ $\Delta Abs_0 = \Delta$ Abs of the blank

Ra =(ratio) = B/B0

logit = ln (Ra/(1-Ra)) B<B0

(2) $\ln(conc) = K \cdot logit^2 + L \cdot logit + M$

K,L and M factors were evaluated using the following matrix equation:

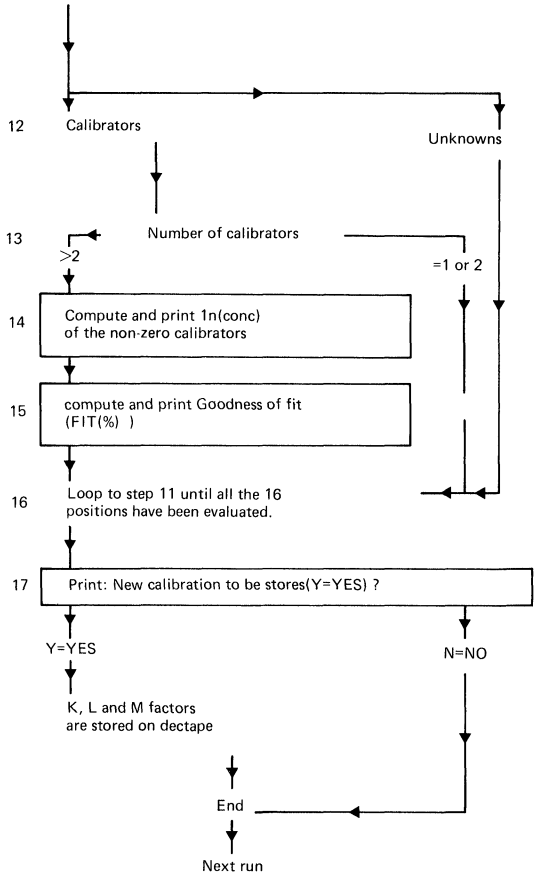


Figure 1.

$$(3)\quad \begin{bmatrix} \sum_{i=1}^{n} \text{logit}_i^2 & \sum_{i=1}^{n} \text{logit}_i & n \\ \sum_{i=1}^{n} \text{logit}_i^3 & \sum_{i=1}^{n} \text{logit}_i^2 & \sum_{i=1}^{n} \text{logit}_i \\ \sum_{i=1}^{n} \text{logit}_i^4 & \sum_{i=1}^{n} \text{logit}_i^3 & \sum_{i=1}^{n} \text{logit}_i^2 \end{bmatrix} * \begin{bmatrix} K \\ L \\ M \end{bmatrix} = \begin{bmatrix} \sum_{i=1}^{n} \ln(\text{conc})_i \\ \sum_{i=1}^{n} \text{logit}_i \cdot \ln(\text{conc})_i \\ \sum_{i=1}^{n} \text{logit}_i^2 \cdot \ln(\text{conc})_i \end{bmatrix}$$

$$(4)\quad \text{conc(unknown)} = e\,(K \cdot \text{logit}^2 + L \cdot \text{logit} + M)$$

The curve fitting procedure was carefully inspected for falsely computed results from sera containing very low drug concentration. The logit-log parabola may reverse "direction" if B is close to BO.

K,L and M factors were stored on dectape in order to check the stability of the calibration between runs. During the first part of the program the calculation is carried out using the stored K,L and M factors in equation 4. If there is an unacceptable deviation from target values, e.g. when only standards are analysed, the curve fitting factors have to be recalculated. Three different modes of recalibration may be performed.

Table 1. Complete calibration using five standards on each disc.

Assay	Sample	Target range/ value µmol/l	Found Mean µmol/l	Stand. dev. µmol/l	CV(%)
Carbamazepine	Seronorm* Pharmaca	63	59.8(n=36)	2.67	4.5
	Syva's aed-control	25.4	25.0(n=52)	0.83	3.3
	unknown	–	4.1(n=7)	0.30	7.3
Ethosuximide	Syva's aed-control	532	558.6(n=18)	18.5	3.3
	Seronorm Pharmaca diluted 1:2	210	215.5(n=36)	10.8	5.0
Phenobarbital	Syva's aed-control	129.2	128.9(n=42)	4.74	3.7
	unknown	–	107.7(n=9)	4.20	3.9
	Autonorm 50*	60-72	75.1(n=35)	0.97	1.3
Phenytoin	Ortho III	83	82.6(n=28)	3.16	3.8
	Syva's aed-control	60	65.6(n=30)	3.67	5.6
	unknown	–	7.3(n=6)	0.77	10.5
Quinidine	unknown	–	24.2(n=18)	0.46	1.9
	Autonorm 50	8.5-13.7	13.5(n=27)	0.43	3.2
	Autonorm 50 diluted 1:4	2.1-3.6	3.3(n=36)	0.16	4.8
Theophylline	Ortho III	111	99.3(n=35)	3.36	3.4
	unknown	–	62.5(n=25)	0.80	1.3
	Ortho III diluted 1:5	22	19.0(n=18)	0.74	3.9

*Manufactured by Nyegaard & Co., Oslo, Norway.

(A) *Recalibration using one standard in position 3 on each new disc.* (Pos 1=water and pos 2=blank). If a parallel shift of the standard curve has occurred in a new run, the following equations may be used for calculation:

$$(5)\quad \ln(\text{conc}) = K_{\text{stored}} \cdot \text{logit}^2 + L_{\text{stored}} \cdot \text{logit} + M_{\text{new}}$$

The parallel shift may be corrected using the measured data of the standard positioned in cuvet number 3 of the rotor:

$$(6)\quad P3 = \ln(\text{conc})_3 - K \cdot \text{logit}_3^2$$

index number refers to the position of the rotor

$$(7)\quad M_{\text{new}} = P3 - L_{\text{stored}} \cdot \text{logit}_3$$

```
*G

 CUV.  DELTA ABS.      B/B0          LOGIT      UMOL/L
   2      0.3186      1.0000
   3      0.6211      0.5561        0.2251       54.5
   4      0.5414      0.6730        0.7217       23.5
   5      0.5339      0.6840        0.7722       21.5
   6      0.5224      0.7009        0.8515       18.6
   7      0.5379      0.6781        0.7446       22.5
   8      0.4774      0.7669        1.1910       10.0
   9      0.3579      0.9423        2.7931        0.3
  10      0.4664      0.7831        1.2836        8.4
  11      0.4672      0.7819        1.2765        8.5
  12      0.5364      0.6803        0.7548       22.1
  13      0.5024      0.7303        0.9959       14.3
  14      0.5717      0.6285        0.5253       33.0
  15      0.4876      0.7520        1.1090       11.6
  16      0.4954      0.7405        1.0485       13.0

 RECALIBRATION (Y=YES)? Y
ENTER NUMBER OF STANDARDS : 2

ENTER STANDARD VALUES

 CUV.    3: 84.0
 CUV.    4: 33.6

 OLD CURVE:
 K= -     0.135
 L= -     1.568
 M=       4.358

 NEW CURVE:
 K= -     0.135
 L= -     1.717
 M=       4.824

 CUV.  DELTA ABS.      B/B0          LOGIT      UMOL/L
   2      0.3186      1.0000
   3      0.6211      0.5561        0.2251       84.0
   4      0.5414      0.6730        0.7217       33.6
   5      0.5339      0.6840        0.7722       30.5
   6      0.5224      0.7009        0.8515       26.1
   7      0.5379      0.6781        0.7446       32.1
   8      0.4774      0.7669        1.1910       13.3
   9      0.3579      0.9423        2.7931        0.4
  10      0.4664      0.7831        1.2836       11.0
  11      0.4672      0.7819        1.2765       11.2
  12      0.5364      0.6803        0.7548       31.5
  13      0.5024      0.7303        0.9959       19.7
  14      0.5717      0.6285        0.5253       48.6
  15      0.4876      0.7520        1.1090       15.7
  16      0.4954      0.7405        1.0485       17.7

 NEW CALIBRATION TO BE STORED (Y=YES)? N


 DATA REVIEW?
```

Figure 2. An outprint produced during the performance of a carbamazepine assay. Two standards, two quality controls and ten unknown samples were analysed. CUV 3 = 84 µmol/l: CUV 4 = 33.6 µmol/l; CUV 5 = Ortho III control serum, target value = 33.6 µmol/l; CUV 6 = Syva aed-control, target value = 25.4 µmol/l. The first part of the outprint shows that the stored calibration factors have to be corrected. The correction is shown in the second part.

(B) *Recalibration using two standards in positions 3 and 4 on each new disc.*

The measured data of two standards positioned in cuvets 3 and 4 may be used for recalculation of two of the curve fitting factors K, L and M. We chose L and M factors for correction, as a correction of the K factor gave erroneous results:

(8) $P4 = \ln(conc)_4 - K_{stored} \cdot logit_4^2$

(9) $P3 = \ln(conc)_3 - K_{stored} \cdot logit_3^2$

(10) $P4 = L_{new} \cdot logit_4 + M_{new}$

(11) $P3 = L_{new} \cdot logit_3 + M_{new}$

(12) $L_{new} = (P4 - P3)/(logit_4 - logit_3)$

(13) $M_{new} = P4 - L_{new} \cdot logit_4 = P3 - L_{new} \cdot logit_3$

(C) *Recalibration using three or more standards on each new disc.*

This mode of calibration involves a new computation of all the three curve fitting factors K,L and M, according to formula 3. The calculated dose of each standard point is found using equation 4. Deviations from target values (deviations are expressed in the program as "Goodness of fit" in %) are calculated in percentages of the target values:

Calculated dose = D_{calc}

Actual dose (target value)= D_{act}

(14) Goodness of fit (%) = $100 \cdot (D_{calc} - D_{act})/D_{act}$

The flow chart of the computer program used is outlined in Figure 1.

Results and discussion

The pipetting station, the loader, was carefully optimised before setting up EMIT runs. It was extremely important to prime the pump syringes prior to analysis. Table 1 shows the precision data obtained when five standards on each disc were used to calculate the standard curve. It is apparent that the coefficients of variation for the EMIT assays studied in this work were low. Unfortunately only nine positions were then left free on the disc for the evaluation of unknown samples. As the first section of the program makes no correction of the stored curve fitting factors, it was possible to check the agreement of standard curves between runs. However, results obtained from several runs of standard curves showed that the agreement was poor. Either a correction of the stored "standard curve" or a recalibration using a full set of standards (= five standards) must be performed. As stated in the data handling section, the facilities to correct a stored "standard curve" using measured data of either one or two standards on the disc were included in the program. Using just one standard for recalculation, the parallel shift correction, resulted in unreliable performance of the assay (results are not shown in this paper). However, recalibration using two standards was acceptable, see Table 2. Using this procedure an additional three samples per disc could be analysed, providing a more economical analysis than that performed with a full set of standards on each disc, although the latter was always superior to other modes of calibration. Two long-term quality control studies were made, see Table 3; they demonstrate the routine applicability and reliability of our computerised EMIT procedure.

Figures 2 and 3 show outprints produced during the execution of our program.

```
*G

 CUV.  DELTA ABS.      B/B0         LOGIT      UMOL/L
   2      0.3169      1.0000
   3      0.6652      0.4900  -    0.0400       83.1
   4      0.6099      0.5711       0.2861       49.3
   5      0.5731      0.6250       0.5104       33.9
   6      0.5152      0.7097       0.8937       17.3
   7      0.4649      0.7833       1.2846        8.3
   8      0.5377      0.6768       0.7387       22.8
   9      0.5907      0.5991       0.4015       40.7
  10      0.5372      0.6775       0.7419       22.7
  11      0.5282      0.6906       0.8027       20.3
  12      0.5356      0.6799       0.7529       22.2
  13      0.5334      0.6831       0.7678       21.6
  14      0.4881      0.7494       1.0951       11.9
  15      0.5507      0.6576       0.6526       26.5
  16      0.5585      0.6463       0.6026       28.9

 RECALIBRATION (Y=YES)? Y
ENTER NUMBER OF STANDARDS : 5

ENTER STANDARD VALUES

 CUV.    3: 84.0
 CUV.    4: 50.4
 CUV.    5: 33.6
 CUV.    6: 16.8
 CUV.    7: 8.4

 OLD CURVE:
 K= -     0.135
 L= -     1.568
 M=       4.358

 NEW CURVE:
 K= -     0.091
 L= -     1.638
 M=       4.375

 CUV.  DELTA ABS.      B/B0         LOGIT      UMOL/L    LN(C)      FIT(%)
   2      0.3169      1.0000
   3      0.6652      0.4900  -    0.0400       84.8    4.4306      1%
   4      0.6099      0.5711       0.2861       49.4    3.9197  -   2%
   5      0.5731      0.6250       0.5104       33.6    3.5142      0%
   6      0.5152      0.7097       0.8937       17.1    2.8211      2%
   7      0.4649      0.7833       1.2846        8.3    2.1279  -   1%
   8      0.5377      0.6768       0.7387       22.5
   9      0.5907      0.5991       0.4015       40.6
  10      0.5372      0.6775       0.7419       22.4
  11      0.5282      0.6906       0.8027       20.1
  12      0.5356      0.6799       0.7529       22.0
  13      0.5334      0.6831       0.7678       21.4
  14      0.4881      0.7494       1.0951       11.8
  15      0.5507      0.6576       0.6526       26.2
  16      0.5585      0.6463       0.6026       28.6

 NEW CALIBRATION TO BE STORED (Y=YES)? Y


   STANDARD CURVE STORED WITH HEADER  950*G
```

Figure 3. A carbamazepine assay performed with a "full set" of standards on the disc. In this case the stored calibration factors are acceptable. However, the standard curve was recalibrated and the outprint from the curve fitting procedure is shown in the second part.

Conclusions

The EMIT assays of carbamazepine, ethosuximide, phenobarbital, phenytoin, quinidine and theophylline from the Syva Corporation are well suited for the Gemsaec centrifugal fast analyser. Results obtained show that the precision of the data fulfills the criteria for routine clinical chemistry.

By use of the specially designed calculation program for the Gemsaec computer, the manual plotting and evaluation is totally eliminated. No additional computer is required to perform calculation of EMIT results. Standard curves can be stored on magnetic tape and may be corrected by use of measured data of two standards in new runs. Three more samples per disc could therefore be analysed.

A great advantage of the EMIT assays is the enormous time saving as compared to other methods of analysis (GC or HPLC). EMIT enhances the possibility of producing fast reports both during the night and the day for the clinician. In an emergency situation this is a great asset for guiding the treatment of drug-intoxicated patients or underdosed patients.

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Table 2. Recalculation of the stored curve fitting factors L and M (see equation 2) of a "good" standard curve (ΣAbs (FIT (%))=5). Number of standards on each disc = 2

Assay: Theophylline					
	Standards		"Unknowns"		
Stated values µmol/l→	222	55.5	111	27.8	13.9
Run	Found*		Found		
1	222	55.5	108.9	28.0	14.4
2	222	55.5	108.9	26.9	12.1
3	222	55.5	114.5	26.1	13.3
4	222	55.5	117.1	26.3	12.0
5	222	55.5	116.2	26.3	12.7
6	222	55.5	115.4	28.6	13.6
7	222	55.5	113.3	27.3	13.6
8	222	55.5	108.6	27.4	14.1
9	222	55.5	113.8	27.4	13.8
10	222	55.5	117.7	26.6	13.9
	Mean =		112.8	27.1	13.4
	Stand. dev. =		3.17	0.81	0.82
	CV (%) =		2.8	3.0	6.2

** The values of the two standards used for calibration will always equal stated values.*

Table 3. Long-term quality control

					Found		
Assay	Period	Control	No. of obs.	Target µmol/l	Mean µmol/l	Stand. dev. µmol/l	CV(%)
Carbamazepine	June 1978-April 1980	Syva's aed-control	159	25.4	25.0	1.21	4.8
	Sept 1979-April 1980	Ortho III	86	33.6	33.0	1.71	5.2
Theophylline	June 1979-April 1980	Syva's theophylline control	75	83.0	82.3	2.69	3.3
	June 1979-April 1980	Ortho III	73	111	105.1	4.10	3.9